1.0 General Information

Fish assemblage monitoring is an integral component of the Oklahoma Water Resources Board (OWRB) Water Quality programs. Assessment of the fish assemblage measures the structure and function of the ichthyofaunal community to evaluate the integrity of a stream. Following is a detailed description of sampling procedures. Efficiency is the key, and finding a comfortable sequence of sampling is essential. This will vary from person to person and from sampling team to sampling team. Yet, employing consistent sampling patterns at every site will maximize the number of sites sampled per day and decrease the chance for introduction of sampling error.

2.0 Definitions/Terms

- Team Leader—crew member of fish collection team who provides support, expertise, and opinions; gives instruction and has final say on how work will be done; must score a 95% on critical fish identification
- Team Member—crew member of fish collection team who provides support, expertise, and opinions; follows the instructions of the team leader
- Formalin—fixative used in fish collections; is a carcinogen and can also cause permanent damage to mucous membranes and eyes.
- Hoop Netting—placement of various sized nets in medium to deep water; selective for areas of instream cover as well as larger fish with more surface area
- Seining—wide and deep net dragged through water to collect fish; selective for runs and pools as well as smaller fish with less surface area

3.0 Safety

Upon reaching the sampling location, site safety determinations should be made before proceeding. Please refer to the OWRB safety manual for instructions. Fishing in non-wadeable streams involves entering water that may be deep and fast or have unseen structure. Care should always be taken when wading into deep areas. General safety guidelines include:

- Primary responsibility for safety rests with the team leader.
- **DO NOT CHASE FISH!!**
- All crewmembers should receive training in First Aid and CPR.
- Always test water for depth and unseen structures.

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1 Much of this SOP is adopted from one document as prepared by Oklahoma State University, Oklahoma Cooperative Fish and Wildlife Research Unit: Oklahoma Water Resources Board (2004): Final Report: Rapid Bioassessments Protocols for Non-Wadeable Rivers in Oklahoma, Oklahoma City, OK.
• Never enter water that is flowing at an unsafe rate. Remember that slippery or loosely packed substrates may compound the effects of swiftly moving water. If you have any doubt about the safety of an area in the river, consult with the team leader. Never enter an area that is outside your personal comfort level.
• Never enter deep water alone.
• Formalin is a carcinogen and can also cause permanent damage to mucous membranes and eyes. Care must be taken when placing fish in formalin so that the fish does not flop around and splash formalin onto people near the jar. Proper precautions should be taken when handling formalin.
  o Protective gloves and eyewear should be worn
  o Avoid inhalation of vapors
• FAILURE TO OBSERVE SAFETY PROCEDURES WILL RESULT IN DISCIPLINARY ACTIONS INCLUDING PROBATION AND DISMISSAL.

4.0 Quality of the Measurement

4.1 Training

Principle investigators for the OWRB are required to have degrees and/or experience with biological or other applicable sciences. Principle investigators are defined as crew leaders, and this designation may be made upon the leader of a multi- or a one person crew. Training is required for all SOPs dealing with water quality and quantity collections and measurements as well as habitat assessments and biological collections. In-house training will be conducted for the use of all meters and digital titrators used for water quality or quantity measurements. Investigators must be familiar with OWRB SOP document and all training will follow the methods outlined in that document. Extra training will be provided when new SOPs are developed. Training of field crews will be done through dry run exercises in the laboratory to familiarize field crews with sample collection, sample preservation, instrument operation, calibration, and maintenance. In addition, when new personnel are hired or new methods developed, qualified staff will train on sample collection, measurement, and field analysis methods through side-by-side field trips. These trips will familiarize staff with SOP requirements. When training is considered adequate, a qualified staff member will check field staff for adherence to SOPs. Prior to collecting fish, all staff should familiarize themselves with Fisheries Techniques (edited by L.A. Nielsen and D.L. Johnson and published by the American Fisheries Society 1983), this SOP, and OWRB Technical Report 99-3 Standard Operating Procedures for Stream Assessments and Biological Collections Related to Biological Criteria and Development.

Investigators are tested for identification abilities with a statewide assemblage of fish fauna before fish collections begin. These fish are comprised of species that are typically found in Oklahoma stream systems. The majority of the test specimens include fish with larger body sizes that are typically field identified and/or found in large numbers. Species of special concern such as the Arkansas River Shiner are also utilized during the testing procedure to ensure endangered or threatened species may be correctly identified and released. A test score on critical species of 95% or better
must be achieved before the investigator will be a field crew leader. Investigators that score under 95% will not collect without direct supervision of the crew leader.

4.2 Kinds of Quality Assurance Samples
4.2.1 Replicate Collections

Replicate samples will be collected during each biological season. The scope and number of replicates will be determined by the project Quality Assurance Project Plan. They may include replicates for various habitat or stream order.

4.2.2 Vouchers and Photodocumentation

The OWRB has adopted a list of critical species (developed by the Oklahoma Conservation Commission) that will be released in the field. This list includes large fish (>100 gms or 0.25 lbs), fish easily identified (e.g., Longnose Gar), and fishes of special concern (e.g., Leopard Darter). However, proper procedures must be taken before fish may be released. Please follow these guidelines:

1. If all team members agree on the identification of such a fish, it can be returned to the water far enough away that recapture is unlikely. **The crew leader does have the final say on releases.**
2. All released fish must be documented on the Fish Collection sheet. Included with this documentation should be the common name (species if known) and characteristics noted to make a positive identification. Fish not recorded, must be brought to the laboratory for identification.
3. All large fish (e.g., smallmouth buffalo) or fish easily mistaken for another (e.g., river carpsucker) must be photographed. Background data in the photo should include the common name of the fish, the date and time (24h), station name or number, and county. Characteristics used to make the identification should be prominently displayed in the photo (e.g., later line scales of a redhorse). Photos should be managed digitally in the OWRB network and printed as needed.
4. When feasible, a voucher specimen should be kept of all released species. This may not be possible with large fish such as suckers or gar. Vouchers should also be kept when a juvenile member of the species is released (e.g., largemouth or spotted bass).

5.0 Personnel and Equipment
5.1 Personnel

Fish collection crews will consist of two to three people. In some instances, a fourth crewmember may be added on larger streams. The team will consist of a team leader and one to three team members. The team leader is someone with one or more seasons of collection experience who has scored above a 95% on critical species identification. Collection experience in other programs may be substituted for that with the OWRB. In certain instances, a team leader may have test scores below 95%. In this case, tests will be reviewed and species that were commonly missed in the scoring
will be excluded from releasing. The team leader will have the final say on all crew activities. A team member is someone trained on fish sampling protocols. Team members will be expected to participate in the decision-making and follow the team leaders direction.

5.2 Equipment and Supplies
5.2.1 Hoop Nets

Various sized hoop nets may be used to collect fish. Recommended sizes vary from 2 to 4 foot diameter nets with 4 to 6 hoops and ½ inch to 2 inch mesh. Hoop size is dictated by water depth, and length and mesh size are determined by fish being targeted. When in place, nets should be under the water and extended fully. To best target fish they should be placed near in-stream structure with the open end facing the structure being targeted. When placing the net, the cod end should always be secured with a zip tie and tied off so that fish cannot escape, and the open end facing downstream and tied to the structure being targeted. In general, the OWRB uses 2 and 3 foot diameter nets with 6 hoops and 1 to 2 inch mesh.

The hoop selects for habitat and fish differently than does the seine. It selects for fish with more surface area (i.e., larger or deep-bodied) such as bass or suckers. Also, the hoop net is more effective in habitat where seining may be more difficult such as deep water and heavy structure. For hoop nets to be most effective they should be used in association with instream structure and in deeper waters. They should also be in place for 12 to 24 hours and left overnight.

5.2.2 Seine

Various sized seines may be used to collect fish. Recommended sizes vary from 3 to 6 foot seines in 10, 20, and 30-foot lengths. Seine height is dictated by water depth, and length is determined by width of the water being sampled. If possible the seine should be 15-25% longer than the width of the waterbody being sampled and about 25% higher than the depth of the water. This will allow the center of the net to form a bag behind the operators where the fish are more likely to stay in the net. However, it is important to remember that the longer the seine is, the more difficult it will be to control in stream currents. Therefore, rule of thumb for length may be discarded. When this occurs, extra time should be spent seining the missed habitats. In general, the OWRB uses 10X6 and 20X6 nets made of 44 pound test (heavy delta) line with no dye or protective coating. A seine 20X8 may be used in very deep and/or long pools. Seines should be a 3/16 inch mesh to reduce fishing pressure on some young of the year.

The seine selects for habitat and fish differently than does the hoop net. The seine selects for fish with less surface area (i.e., terete or smaller body plan) such as minnows or darters. Also, the seine is more effective in habitat where hoop nets may be ineffective such as shallow areas (< 0.75 meters) or backwater pools. Seining may also be done along banks and around instream cover. Seining technique is explained in the later methods section. Seines should be stored dry and free of debris and other snags.
5.2.3 General Supplies

Clothing
- Rubber Gloves for use in mixing formalin
- Goggles for use in mixing formalin

Documentation
- Dry erase board or white paper and clipboard for photodocumentation
- Camera with at least 2 rolls of film or adequate digital memory
- Tape measure or measuring board to record lengths of released fish if desired

Chemicals
- 10% buffered formalin—try to premix in lab and keep jugs stored in separate ice chest

Nets
- Fishing line, cord, or zip ties to repair nets
- Extra ¼ inch braided nylon cord for setting hoop nets
- Zip ties for tying cod end of hoop nets

Containers
- Wide mouth 1-gallon jars, at least 4 per site
- Quart jars for special fish or paratype collection specimens

6.0 Collection of Fish

The collection of fish follows a modified version a Rapid Bioassessment Protocol developed by the Oklahoma State University, Oklahoma Cooperative Fish and Wildlife Research Unit, the Field Operations Manual of the USEPA’s Western EMAP Pilot Study (EPA, 2000) and is supplemented by other documents. Specific techniques for, and relative advantages of seining and hoop nets vary considerably according to river depth and in-stream structure. The specifics are discussed in detail in Fisheries Techniques (edited by L.A. Nielsen and D.L. Johnson and published by the American Fisheries Society 1983).

The collection of fish involves the use of two collection methods, seining and hoop netting. The combination of methods was selected in order to produce a representative fish collection. Variations of habitat, type of fish, and water chemistry (exclusion of electrofishing) dictate the use of different collection techniques. Hoop nets select for denser habitat (e.g., wood piles, root wads, etc.) and deeper habitat while seining selects for more open habitat such as runs or pools, backwaters, and shallower areas. Hoop nets select for more surface area (e.g., larger or deep-bodied fish) while seining selects for smaller fish such as minnows darters. Hoop nets may be ineffective in waters < 0.75 meters deep. Seining may be ineffective in waters with heavy structure.
Sequence when using both methods is determined by site characteristics. In general, hoop nets should be placed the night before seining so that fish that are displaced may resettle. Since most hoop netting will be done overnight, seining may be done in the early morning to early afternoon hours. Seining should always be done before harvesting hoop nets because access to hoop nets is often through shallow waters that will be seined. Backwaters may be seined at any time.

As a general rule, each stream is sampled for a distance of 1000 meters. A representative stream reach is selected and measured so that primary physical features are included in the reach. These include heavy structure, backwaters (when present), slow and fast areas, shallow and deep runs, and shallow and deep pools. Extremely large rivers may be sampled at a length greater than 1000 meters if all representative habitats are not represented in a 1000 meter reach. Again, ensure that all available habitats are represented in that reach. The reach should be located away from the influences of major tributaries and bridge/road crossings. Record reach length on the Field Notes.

In general, all fish are placed in 10% formalin immediately after capture. However, if larger fish (> 100 g) can be positively identified in the field, they are returned to the water in a location where recapture is unlikely. All large fish that may be easily confused with a close or related species are photographed on print film before release. A representative photograph or voucher specimen may be taken when large numbers of one fish species is collected and released. In all instances when fish are released, they should be recorded and the characteristics used to make the identification should be noted. Collected organisms are identified to species by an experienced taxonomist.

6.1 Mapping of Sampling Areas

A major prerequisite of any RBP is the apportionment of time or effort during collection. The primary goal is not a census of the available community and its populations but to determine the general health of the biotic community. By applying a consistent unit of effort to collections across all sampled sites, general assumptions can be made about reference and non-reference conditions, and subsequent criteria or indices of biological integrity can be developed.

Because of the sheer numbers and sizes of available habitats in non-wadeable streams, an entire reach cannot be sampled with efficiency. Fortunately, the studies used to develop non-wadeable RBP’s have shown that an acceptable number of total species may be caught using certain gear with an appropriate unit of effort. As discussed previously, the gear developed for prairie non-wadeables are the seine and the hoop net. Furthermore, each gear type has a particular unit of effort associated with it that will maximize the catch potential for the available species.

For prairie non-wadeables, habitats to be sampled are apportioned using the prairie non-wadeable habitat assessment form (refer to Prairie Non-wadeable Habitat SOP). Reach wide habitat is measured using twenty (20) equidistant transects (50 meters
Each transect measures a number of characteristics to determine certain features. These measurements consist of two groups: transect-wide measures and transect-only measures. Transect-wide measures include quantification of instream habitat, bank stability, and riparian condition, among other things. Transect-only measures include quantification of wetted and bankfull widths, depths (right and left wetted edge, right and left quarter, and center), substrate percentages, and habitat types described in Table 1. To increase resolution for transect-only measurements, these measurements are made at an additional nineteen (19) mid-transect cross-sections. Depth and habitat characterization are then used to apportion both gear and unit effort. The apportionment is described in sections 6.2 and 6.3 of this document.

**TABLE 1:** Description of the habitat types used when mapping the sample reach.

<table>
<thead>
<tr>
<th>Habitat Type</th>
<th>Code</th>
<th>Depth (m)</th>
<th>Velocity (m/s)</th>
<th>Channel Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shallow Slow</td>
<td>SS</td>
<td>&lt; 0.75</td>
<td>&lt; 0.20</td>
<td>Typically found along the bank or around mid-channel islands.</td>
</tr>
<tr>
<td>Shallow Fast</td>
<td>SF</td>
<td>&lt; 0.75</td>
<td>&gt; 0.20</td>
<td>Typically found mid-channel away from obstructions.</td>
</tr>
<tr>
<td>Deep Slow</td>
<td>DS</td>
<td>0.76 to 1.50</td>
<td>&lt; 0.20</td>
<td>Lateral pools typically surrounded by woody debris (or other structure).</td>
</tr>
<tr>
<td>Deep Fast</td>
<td>DF</td>
<td>0.76 to 1.50</td>
<td>&gt; 0.20</td>
<td>Lateral or mid-channel pools typically free of obstructions.</td>
</tr>
<tr>
<td>Non-wadeable</td>
<td>NW</td>
<td>&gt; 1.50</td>
<td>Any Velocity</td>
<td>Typically found in the thalweg surrounded by very little structure.</td>
</tr>
<tr>
<td>Backwater</td>
<td>BW</td>
<td>Any Depth</td>
<td>&lt; 0.01</td>
<td>Standstill water either mostly or totally separated from the main channel.</td>
</tr>
</tbody>
</table>

### 6.2 Hoop Nets

Hoop netting is a passive fishing technique used to catch larger fish and in areas of deep cover. Generally, they are only effective in water > 0.75 meters deep and will be placed in deep slow, deep fast, and non-wadeable habitats. For maximum efficiency, they should only be placed near in-stream structure such as logjams or woody debris piles. Furthermore, to maximize catch, a mixture of large and small nets should be used. A minimum of 6 (3 habitats) and a maximum of 10 (5 habitats) nets should be placed within each reach. For best results, follow these guidelines:

1. Place nets near in-stream structure (e.g. woody debris, boulders, and undercut banks).
2. The opening of the net should be downstream facing the center point of the structure. It should extend parallel (or underneath if possible) to the structure.
3. If possible, the open end of the net should always be secured to structure and should not be obstructed. If T-posts are necessary, place two posts...
to the sides of the net and tie off to both. The cod end may be tied to a T-post or structure.

4. Always place a zip tie on the cod end ensure the fish cannot escape.
5. Wings may be placed at the sides of the net to increase catch efficiency.

Hoop nets should be placed near the end of the day and left for 12 to 24 hours overnight. Because hoop nets often catch larger fish, nets should only be harvested by two or more people and not opened until out of the water. To avoid loss of catch, injury to personnel, or damage to equipment, dump hoop nets through the cod end on a sandbar or bank. Most fish will be critical species and may be released after proper documentation and/or collection of voucher specimens.

6.3 Seining

Seining is a active fishing technique used to catch smaller fish and in areas of sparse to no in-stream cover. Although seines can be used effectively in slower deeper waters, they are only generally used in water < 0.75 meters deep when collecting in prairie non-wadeables because of the use of hoop nets in the deeper waters. The habitat types to be seined are those designated as shallow fast, shallow slow, and backwater. At least 25% and no greater than 50% of the reach designated as shallow slow or fast should be seined. If these habitats represent less than 25% of the entire reach, all should be sampled. These areas may be seined vertically working downstream or diagonally across the habitat. More than one seine haul may be done in a particular area if the area is unusually wide or the team leader determines that other species may be present. Which areas of the reach are sampled is at the discretion of the best professional judgment of the team leader. All backwaters should be sampled. Depending on the density of debris, one seine haul through each backwater should be adequate. If depth of or debris in the backwater poses a hazard to personnel or equipment, the backwater will not be seined (this is the sole judgment of the team leader). If this situation arises, a hoop net may be placed at the discretion of the team leader. Habitats designated as deep slow may be seined if these conditions exist:

1. Using best professional judgment, it is determined that hoop nets have not been effective at yielding a species composition representative of reach.
2. In-stream debris does not pose a hazard to equipment or personnel.
3. Depth and velocity does not pose a hazard to personnel.
4. Use of seines in these habitats is at the discretion of the team leader.

Seining should not be done in an area immediately after hoop nets are placed or harvested. Although a 10 x 6 foot seine may be used, 20 x 6 foot seines should be the primary gear used unless considerable debris exists. All non-critical fish should be harvested and placed in formalin. Fish designated as critical species should be positively identified, enumerated and released. Proper documentation and/or collection of voucher specimens should be done during each release.

To seine, two people drag the net through the water at a certain rate. This will allow the center of the net to form a bag behind the operators allowing the fish room to move within
the net. Generally, the seine is hauled with the current because fish tend to orient towards the current. The leadline should be kept on the bottom, and in front of the float line. If there are many obstructions on the bottom, the leadline will become caught or bounce, and most fish will escape underneath the bottom of the net. If this happens use a smaller net that allows you to avoid obstructions, roll up the ends of the existing net to make it more manageable, or use a trailer to move net over obstruction. The brailles of the net should be used to disturb the area under any undercut banks, bedrock ledges, or beds of macrophytes near the edge in order to scare fish hiding in cover out towards the middle of the net.

Under ideal conditions the net should be pulled through the water in the manner described above for about 10-20 meters and dragged out of the water on a gradually sloping preselected beach. The person pulling the seine on the side of the stream opposite the beach should swing ahead of the other person so that the seine is pulled out on the beach stretched over the same distance it was stretched in the stream. If the stream doesn't have gradually sloping banks, the dip method should be used. This method consists of sweeping around and through the area to be sampled, keeping a wide bag and moving the lead line as much under the undercut bank as possible. Use the brailles to probe repeatedly as far as possible into the undercut area working towards each other until the brailles overlap. The seine should then be swiftly stretched and lifted vertically from the water.

Other seining methods may be effective. All will not be discussed here, but may be demonstrated in the field. In certain instances moving with the current may not be possible. In these cases to keep from loosing fish, the bag should be deepened and the total reach seined should be shortened. Also, it is often not possible to reach a seine the width of a run while keeping an adequate bag. In these cases, seine perpendicular to the flow of the water towards the opposite shore. The downstream person should operate slightly ahead of upstream person forming a “J”.

RECORD THE TIME SPENT SEINING AND SEINE MESH SIZE ON THE FIELD DATA SHEETS.

6.4 Sample Handling & Preservation

CAUTION: Formalin is a carcinogen and can also cause permanent damage to mucous membranes and eyes. Care must be taken when placing fish in formalin so that the fish does not flop around and splash formalin onto people near the jar. The fish should be put into the jar with the lid tilted open away from the operator so that the lid shields the face and body of the operator. Flood any skin exposed to formalin with plenty of water as soon as possible. If it gets in your eyes, flood the eyes with water immediately and go to the doctor immediately after that.

The following steps should be taken to handle and preserve fish:
Fish collected by seining and electroshocking should be kept in separate jars and labeled with capture method. This will make the methods independent if desired for analysis.

Label each jar. Using a pencil, write the date, WBID #, collection time, stream name, number of jars composing one sample, county, legal location, and crew leader’s name on a piece of ~2 x 3 inch waterproof paper and place one label into every jar of fish from each site. Write the same information on the lid and the front of each jar using a wax pencil or an indelible marking pen.

In general all fish should be placed in 10% formalin immediately after capture. There are a few exceptions made for larger fish (>100 gms or 0.25 lbs), which can be positively identified in the field. If all team members agree on the identification of such a fish, it can be returned to the water far enough away that recapture is unlikely.

All released fish must be documented on the Fish Collection sheet. Included with this documentation should be the common name (species if known) and characteristics noted to make a positive identification. Fish not recorded, must be brought to the laboratory for identification.

All large fish (e.g., smallmouth buffalo) or fish easily mistaken for another (e.g., river carpsucker) must be photographed. Background data in the photo should include the common name of the fish, the date and time (24h), station name or number, and county. Characteristics used to make the identification should be prominently displayed in the photo (e.g., later line scales of a redhorse). Photos should be managed digitally in the OWRB network and printed as needed.

When feasible, a voucher specimen should be kept of all released species. This may not be possible with large fish such as suckers or gar. Vouchers should also be kept when a juvenile member of the species is released (e.g., largemouth or spotted bass).

When preserving fish much larger than 0.3 to 5 kg (0.5 to 10 lbs), the fish should be sliced open along the lower rib in order to allow the formalin to penetrate the body cavity fast enough to prevent decay. A slit through the ribs is preferred to a belly slit to facilitate counting belly scales in the lab.

Fill out a Chain of Custody Form.

The Crew Leader is responsible for transferring the samples to the Fish Sample Taxonomist (currently the OU Museum of Natural History).

7.0 Forms

7.1 Field Notes
Field notes are documents used to annotate and record information that is gathered at the project site. They are a data sheet and should be treated as such. Therefore, they should be written, legible, and complete. To avoid confusion and loss of data, a new sheet should be used at each new project site. Field notes should be initialed and dated by the collecting personnel and data entry personnel. For guidance on proper procedure to complete the field notes, refer to your supervisor and or FTE. Field notes can be found at S:\Monitoring\STREAMS\forms\.

7.2 Laboratory Log-in Sheets
Release sheets are documents used to identify and enumerate all released fish. Fish should be identified with the full common name and scientific name if remembered. Each fish released should be denoted with a tic mark. If a representative individual(s) has been photodocumented or kept as a voucher species, this should be noted in the appropriate column. All characteristics used to identify the fish should be noted in the comments field. For example, if releasing a largemouth bass, the comments section should discuss the separation of the spinous and soft dorsal, how far the jaw extends past the eye, presence of a lateral band, presence/absence of a tooth patch, etc. The form is a data sheet and should be treated as such. Therefore, it should include the date, collectors, and gear used and be legible and complete. To avoid confusion and loss of data, a new sheet should be used at each new project site. For guidance on proper procedure to complete the log-in sheets, refer to your supervisor and or FTE. Log-in sheets can be found at S:\Monitoring\STREAMS\forms\.

7.3 Chains of Custody
Chains of custody are documents turned into the analytical laboratory for each group of samples collected. These forms are used for several purposes. They act as a legal document to show proper delivery of samples occurred and they make a general list of the parameters that should be analyzed. They are a data sheet and should be treated as such. Therefore, they should include the date, collectors, and gear used and be legible and complete. They should also be signed and dated by field and laboratory receiving personnel at the time of delivery. To avoid confusion and loss of data, a new chain of custody should be used for each delivery. For guidance on proper procedure to complete the chains of custody, refer to your supervisor and or FTE. Chains of custody can be found at S:\Monitoring\STREAMS\forms\.

8.0 Data Storage
All completed paper copies of forms and data sheets should be maintained with the appropriate station notebook. The data from the field notes and laboratory data sheets should be either entered into or uploaded to the Water Quality Biological Database. Each sample should be maintained electronically in the database under a unique sample number.

9.0 References

