Chapter VI. FISH COLLECTION PROTOCOLS – WADEABLE STREAMS

Overview

Fish as Environmental Indicators

Fish community assessments are an important component of many water quality management programs. These assessments are useful for making decisions in regard to aquatic life use-support designations, biological integrity, consumption advisories, and overall stream health. There are several advantages of using fish as indicators of biological integrity:

- Fish are long-lived and mobile, thus they serve as good indicators of long-term effects and broad habitat conditions.
- Fish assemblages generally represent a variety of trophic levels (omnivores, herbivores, insectivores, planktivores, and piscivores) and are reflective of overall stream health.
- Life history and distribution information of most fish are well known.
- Fish are relatively easy to collect and identify to the species level.
- Fish are at the top of the aquatic food web and are consumed by humans, making them important for assessing contamination.

Basis of Sampling Method

Sampling methods used in the WVDEP-Watershed Assessment Branch (WAB) are qualitative in nature and essentially derived from USEPA – EMAP protocols with some deviations (Lazorchak, J.M., Klemm, D.J., and D.V. Peck (editors) 1998. *Environmental Monitoring and Assessment Program – Surface Waters: Field Operations and Methods for Measuring the Ecological Condition of Wadeable Streams*. EPA/620/R-94/004F. U.S. Environmental Protection Agency, Washington, D.C.). These methods are widely accepted and used by many states and agencies, each usually with their own specific alterations to better meet their individual needs. However, it is important to note that consistency in regards to methods, time/effort expended, and overall sample collection is critical for obtaining comparable assessments. In general, the methods involve the use of a device capable of generating an electric current, usually a backpack electrofishing unit.

Currently, the main objective of fish community assessments for WVDEP-WAB is to collect data from random (probabilistic) and targeted sites (***refer to Chapter II. Section A. Accessing the Site starting on page 8 for a description***) that can be assessed with a fish based multi-metric index (MMI) or Index of Biotic Integrity (IBI). Primarily, sampling is focused on randomly selected sites chosen each year as part of a five year rotating statewide cycle. Ten sites are selected from each of three ecoregions (Western Allegheny Plateau, Central Appalachians, Ridge and Valley) within the state. Five of the ten sites are new (not previously visited) and five are repeat sites that were previously sampled in the last cycle. WAB began collecting fish community data in 2006, therefore no fish sample sites will be true repeats until 2011.

Beginning in 2011, additional fish samples will be collected from LTMS (Long Term Monitoring Stations) and statewide AWQN (Ambient Water Quality Network) locations. Approximately ten sites will be sampled each year in a three year cycle and then re-sampled in the next three year cycle. Long-term sampling of these sites is important in establishing trend data and in making observations on variability in the fish community. Data from sites that meet sampling suitability criteria (explained below) will be assessed with an MMI/IBI. Currently (April 2011), WAB does not have a fish MMI/IBI developed for use with fish community data. However, data collection is the first step in the MMI/IBI development process.

Part 1. Selecting Sampling Sites

Sites will be selected and sampled based on the following criteria that will produce comparable data that can be assessed using an MMI/IBI.

1. The stream reach must be wadeable. Wadeable streams are those that can be safely waded while electrofishing with either a backpack electrofisher or tote barge and allow the shocker and netter to reach all available habitats. Exceptionally deep pools or deep/fast runs may be omitted or sampled with alternative methods. Ultimately, a careful, concerted effort must be made to sample as much of the reach as possible using comparable methods.

2. Watershed size for a selected site is between 2,000 and 100,000 acres which encompasses some first order up through fourth order streams. The minimum size was selected to exclude the smaller streams which may be limited to one or two species or no fish at all and the maximum size corresponds to streams sites exceeding 100,000 acres, which are typically too large to be considered wadeable due to morphology and /or ecoregion characteristics (long- deep pools, water turbidity, etc.). Additionally, this maximum size has been used by researchers as the upper size limit for some fish MMI/IBI development projects.

3. Assessments will be conducted from mid-May through the end of October which will be considered the Index Period. Initial focus will be on small (1^{st}, 2^{nd} order) streams and progress to larger streams later in the year when lower flows allow for easier sampling. In general, 3^{rd} and 4^{th} order streams should not be sampled until mid-June or later. Most importantly, all sampling should occur during normal flow conditions.

Other types of sites or sampling related to special projects (fish kills, stream restoration, trout surveys, etc.) may or may not allow strict adherence to these criteria due to needs of the project.

Part 2. Determining Site Suitability

Many of the sites selected (primarily randoms) for fish community assessment will be visited by a sample team to collect benthic macroinvertebrates prior to the fish collection visit. This team should make observations and record notes regarding the suitability of the site for fish collection. The notes should contain information pertaining to flow status (*e.g.,* too deep, possibly dry later in the summer, etc.), site access (*e.g.,* landowner issues, limiting physical barriers), and stream morphology that could influence the sampling effort (*e.g.,* large pools or falls). Notes should be given to the fish crew prior to the site visit. Thus, if conditions exist that would prevent a comparable fish collection the fish crew could avoid a costly trip to the site.

Some sites will be selected for long term temperature monitoring in order to determine their summer maximum. Ultimately, the temperature information may be used to assess whether the fish community at a particular site is representative of warm, cool, or cold water conditions. This may also be important in the development of a fish MMI/IBI. These sites will be visited by members of the fish crew in the spring for placement of deployable temperature units. At this time, crew members can also make observations on the suitability of the site for fish collection.

In order to determine if a site can be sampled, the fish collection crew leader should examine the entire proposed reach upon arrival before any sampling occurs. The primary factors to consider when determining if a reach can be effectively electrofished are safety, available habitat, and flow status. In addition, the crew leader should consider abnormal or unnatural features (*e.g.,* bridges, culverts, etc.) that may be present in the reach. Some features may not prohibit sampling if adjustments are made properly. For example, if a small, short culvert is present within the reach, the culvert length should be measured and that distance added to the upper end of the reach. Subsequently, the culvert can be simply omitted from the sample area. Possible conditions that would prevent sampling are a dry stream channel, dense overhanging vegetation that prohibits efficient movement and/or collection, and above normal flows. Under no circumstances should a stream be sampled if dangerous conditions are present.

Part 3. Establishing the Sample Reach

The length of the sample reach will be 40 times the average wetted width of the stream, with a minimum length of 160 m and maximum length of 500 m. The average wetted width is determined by taking three to five measurements (based on variability) within a reasonable distance (~100 m) from the x-site. This should be done in an upstream direction if the X-site is at the downstream terminus of the reach. It should be done both upstream and downstream if the X-site will not be used as the downstream terminus during the fish collection.

The sample reach should include all available habitat types. The various habitat types that may be encountered are defined as follows:

Pool - Still water with low velocity. Water surface is smooth and glassy. Usually deep compared to other parts of the channel.
Glide - Slow moving water with a smooth, unbroken surface. Turbulence is low. Usually shallow compared to other parts of the channel.
Run – Similar to glide but water is moving slightly faster. Turbulence is low and the surface is without ripples that produce gurgling sounds. Runs may have small waves.
Riffle - Water moving with small ripples, waves and eddies. Produced a babbling or gurgling sound.
Snag - Submerged woody debris (dead logs, root wads, etc.).
Submerged Macrophytes - Aquatic vegetation growing beneath the water surface.
Vegetated and Undercut Banks - Stream banks having submerged vegetation (shrubs, etc.) and/or root wads.

If possible, the lower and upper end of the reach should be located at or near some type of hydraulic feature (*e.g.,* riffle, plunge pool, etc.) which will serve as a barrier to fish movement. If no barrier is located at the ends of the reach, then block nets or seines should be used to corral and contain fish during electrofishing process.

All sample locations should be chosen based on these criteria. Any deviations should be thoroughly documented so that a determination can be made as to whether the sample is comparable for MMI/IBI purposes.

Section A. Fish Sampling

The number(s) and type(s) of sample gear will be determined based on stream width and morphology. Experienced professional judgment is critical in this determination. The goal is to be confident that the fish community is being adequately and thoroughly assessed.

Materials and Reagents

1. <u>Electrofishers</u> - Smith-Root Model 24LR or Model 12 backpack electrofisher
2. <u>Electrofisher batteries and chargers</u> – Spare batteries should be handy and available to ensure that a site can be electrofished quickly
3. <u>Electrofisher cathode and anode</u>

4. Tow barge – Includes a generator, anode pole and cable, GPP electrofisher, and cooler, and fuel.
5. Dipnets - 1/4" mesh; assorted frame sizes
6. Seines/blocknets - 1/4 in. mesh; 4'x20' or 4'x30' dimensions
7. 1 gal. Nalgene jars
8. 37% Formaldehyde
9. Assorted plastic buckets with lids – Used to hold fish between capture and field processing
10. Sample Jar Identification Labels - For both inside and outside of the jar
11. Chest Waders – Waders should not be breathable in order to prevent accidental electrical shock
12. Rubber Gloves – To be worn at all times by electroshockers and netters
13. Measuring board and digital scales
14. 100 meter tape measures – At least 5
15. Polarized sunglasses
16. Hearing protection – Used when using the tow barge/generator
17. Fish collection form and WAB field assessment form
18. Digital camera – Used to document large fish that will be released (*e.g.,* large game fish or rare, threatened, or endangered species) and fish health anomalies
19. GPS
20. Scientific collecting permit – Obtained yearly by Watershed Assessment Branch from the WVDNR.

Field Safety Precautions

Formaldehyde is a known human carcinogen! The vapors and solution may cause severe irritation upon contact with skin and eyes. Use caution when handling and wear nitrile gloves and eye protection. If indoors, always work in a well-ventilated area.

Safety methods and protocols can be referred to in the following documents:

Professional Safety Committee. 2008. *Fisheries safety handbook*. American Fisheries Society, Bethesda, Maryland.

User's Manual. *GPP 2.5, 5.0, 7.5, and 9.0 Portable Electrofishers*. Smith-Root, Inc. Electrofishing Safety and Principles section, pgs. 14 -18.

All electrofishing crew members must read and be familiar with these safety protocols. In addition, anyone participating in an electrofishing activity will be required to read and sign the "Acknowledgement of Electrofishing Orientation" form found on page 19 of the American Fisheries Society safety handbook.

Part 1. Sample Collection Methods

Before sampling event:

- Fill out sample labels with a No. 2 pencil. Attach to the outside of the sample jar using clear, waterproof tape. Fill out a pre-printed sample label made of waterproof paper for the inside of the sample jar.
- Fill the sample jar 1/5 full with 37% formaldehyde.
- Check all of the nets to ensure there are no holes. If there are holes or tears in the net, it should be repaired immediately before the next sample is collected and/or replaced as soon as possible.

Electrofishing

Figure 59. An electrofishing crew consisting of two backpack shockers and three netters.

Electrofishing is the primary method of fish collection used by the Watershed Assessment Branch (WAB) in wadeable streams and rivers. It is usually the most efficient and effective method, however other methods such as seining, gill netting, and angling are also utilized. The electrofishing crew consists of one crew leader and a minimum of one other experienced crew member. Normally, there are at least three crew members at each site. If there are additional crew members, they do not have to be experienced, but they must be knowledgeable of the safety and principles of electrofishing. ***See Table 10 below for guidance on the number of electrofishers and netters required for different stream sizes and depths***.

Table 10. Personnel and equipment required to effectively electroshock various types of streams.

Stream Width	Stream Depth	Number/ Type of Electrofishers	Number of Netters
≤ 4m	Shallow (< 2')	1 backpack	2
≤ 4m	Deep (> 2')	2 backpacks	2 – 3
4 – 8 m	Shallow	2 backpacks	3 – 4
4 – 8 m	Deep	2 backpacks or Barge	2 – 4
> 8 m	Shallow	2 backpacks	3 – 4
> 8 m	Deep	Barge	2 – 4

In general, where conditions allow, one backpack electroshocker will be used in streams up to four meters wide. In shallow streams with little or no area for fish escape, one electrofisher working upstream in a side to side motion can adequately shock the majority of the habitat present. In streams four to eight meters wide and in deeper, trough-like streams less than four meters wide (often associated with reduced visibility), two backpack electroshockers will be used (***see Figure 59 on previous page***). The two electrofishers will parallel each other working side to side in an upstream direction (***see Figure 60 on next page***). It's important to keep the two anodes from getting too close to one another. If the anodes are too close it will tax the system, reduce the effective range, and produce a much larger voltage gradient near the anodes which could be lethal to the fish (refer to the backpack electrofisher manual for further explanation). However, don't allow an excessive distance between the anodes which would create potential for fish to escape.

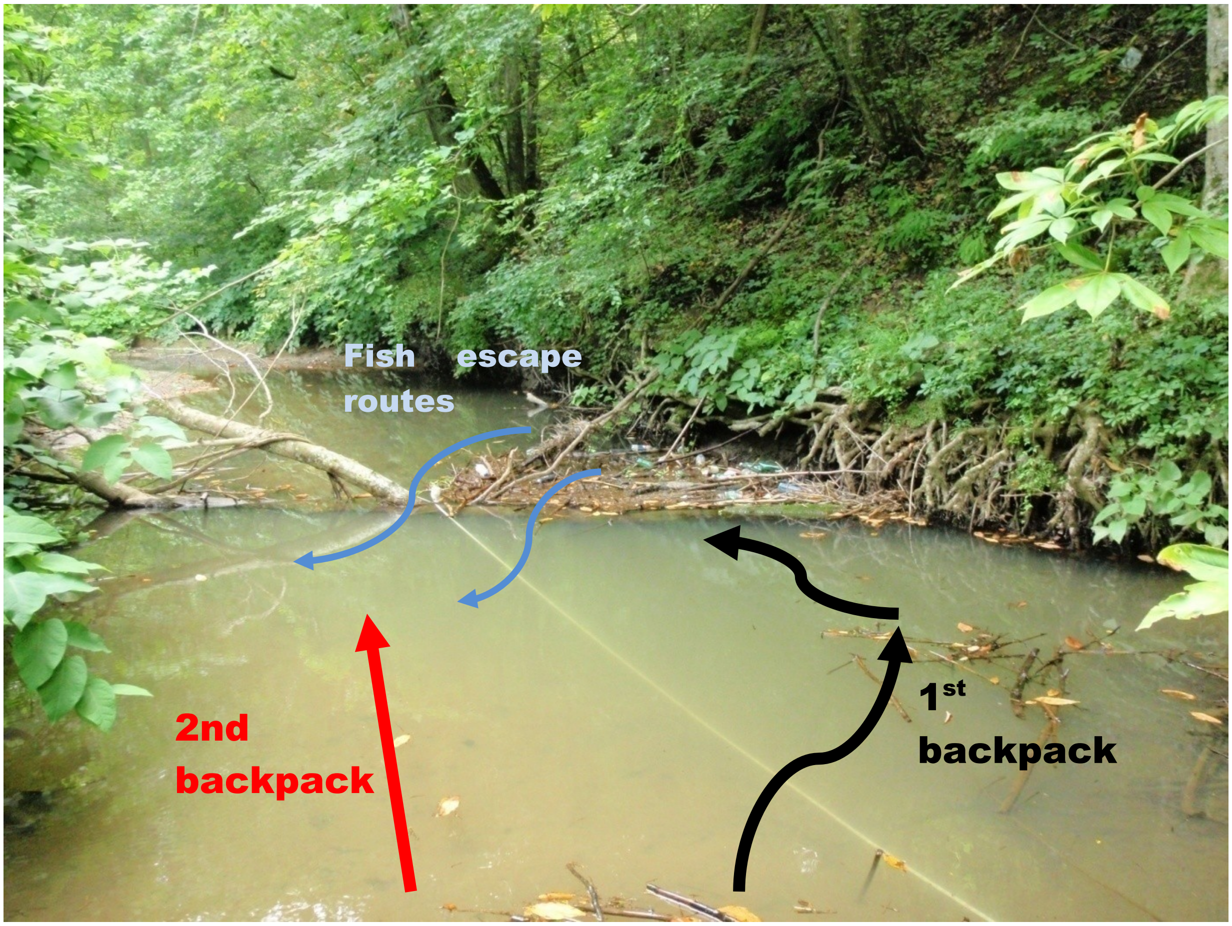

Figure 60. Technique for sampling a deep, narrow stream with two backpacks, looking upstream.

A tow barge ***(see Figure 61 below)*** will be used in streams eight meters wide or greater (assuming that water depth is adequate to move the barge) (***see Figure 62 on next page***). The tow barge offers more power to a much larger sampling area than a backpack electrofishing unit. The barge should also be used whenever possible in water with higher specific conductance (>1,000 µmhos/cm) because a backpack unit is often not capable of producing enough power to adequately stun fish in these conditions. If a stream greater than 8m wide is too shallow to use the barge, then two backpacks should be used. The decision should be made by the crew leader as to whether the backpacks are adequate to obtain a representative sample. If not, then the stream should not be sampled as it would result in a non-comparable sample.

Figure 61. Tow barge with generator, live well, and shocking wand.

Figure 62. Reach type requiring barge electrofisher. Note the large width and lack of constraining features or habitat.

In a stream with multiple or braided channels (***see Figure 63 below***), each channel should be electrofished. Using one backpack, select a channel and shock it upstream to the point where the main channel splits. Walk back down that channel to the starting point and then begin working up the next channel. Continue this pattern until all available channels have been sampled. When sampling a stream with substantial quantities of a specific cover type (*e.g.* boulders, logs, undercut banks, overhanging vegetation), focus your effort on that habitat. Most species of fish tend to hide as a first response to disturbance. Use the anode to draw fish out of the cover where they can easily be netted. If the proper settings are used, the anode will actually work like a magnet and the fish will swim to the anode.

Figure 63. Proper technique for sampling a stream with multiple channels.

One to two netters should accompany each backpack electrofisher and a minimum of two netters (one on each side) must accompany the tow barge. Therefore, a minimum of three crewmembers are required for backpack electrofishing and at least four crewmembers are required for tow barge electrofishing (one shocker, one barge mover, and two netters). In most cases, one or more of the netters can carry a bucket for fish transport and aeration. If an additional crewmember is available, that person can carry the bucket and maintain the fish. This results in added convenience for the netters. When using the tow barge, a cooler or large plastic tub is placed in the barge for fish transport. *IMPORTANT: Focus should be placed on transferring collected fish as often as possible from the nets to the bucket or cooler to reduce mortality rates. Also, any* *fish determined to be voucher specimens need to be placed in sample containers with preservative as often as necessary. Ideally, fish should be preserved while they are still alive to maintain as many distinguishing characters as possible. Dead fish will lose their color and markings very quickly.*

Netting

Initially, netting fish would seem like a very simple procedure. However, certain guidelines must be followed for the electrofishing survey to be carried out properly and efficiently. These guidelines are as follows:

1. Netters should remain adjacent or slightly behind the backpack shocker. Netters moving ahead of the shocker will not only frighten fish away, but will be out of position to net fish as they float behind the shocker. Equally as important, the netter(s) should not trail too far behind the shocker to allow the fish to recover and swim away. The goal is to stun the fish temporarily so they can be netted quickly. All netters should be aware of the effective electrofishing field so they can anticipate where fish will float to the surface of the water.

2. Keep the net at or just below the surface of the water while moving upstream. Fish can often float by very quickly or dart around the net if they are not completely stunned. The closer the net is to the fish and shocking area, the more likely the fish will be captured. Do not carry the net on your shoulder or use it as a leaning post. Be ready at all times.

3. Do not use the net as a shovel; we are not collecting gravel. Fish that are trapped between substrate features can easily be obtained using a simple technique. Place the front of the frame of the net just over the trapped fish. Then quickly pull the net up and away from the fish creating a surge of water toward the surface which should draw the stunned fish up from the bottom (Note: this may take a few attempts) allowing the fish be netted. With practice, the technique can be perfected and proves very effective.

4. If an additional person is available to carry the bucket, that person can also carry a small net to collect any stragglers that the netters have missed. This person should stay a few feet behind the shocker and primary netters.

Seining

When sampling larger streams with deep pools, there are times when electrofishing is not possible or at least not productive (*e.g.* the pool is too deep to wade without submerging the backpack shocker, the stream bottom is unstable, the water is too turbid to see fish, etc.). In these conditions, the sampling crew should attempt a seine haul.

One crewmember stands with one end of the seine on one bank. The seine is then stretched, perpendicular to flow, across the stream and held by another crew member on the opposite end. Either crew member (only one) should then walk upstream or downstream in an arc to the opposite bank keeping the seine moderately taught at all times, but with some slack to form a pocket. After reaching the bank, the seine should be lifted quickly and carefully out of the water and placed on the bank. Fish are then removed and placed in buckets. Based on success of the first haul, a second may be performed, but no more than two sweeps should be performed for consistency. The success of the seine haul is determined by the crew leader or most experienced crew member.

Fish Collection Procedure

1. Prior to or upon arrival at the site, the crew leader should review all available information so decisions regarding time and effort needed to sample the site can be made. Also, all equipment should be checked to make sure it is present and in working condition.

2. For random, AWQN, and LTMS sites, locate the x-site for proper reach confirmation. For non-random sites, simply determine where the reach will begin. Obtain GPS coordinates and verify correct location (***see Chapter II. Section B. Part 1. Coordinates and Global Positioning Systems (GPS) starting on page 22***). If a previous visit was made to the site for macroinvertebrate collection, be sure the coordinates match.

3. Collect appropriate water quality samples (*e.g.,* fecal, metals, nutrients, etc.) and field meter parameters. It is important to note that no one should enter the stream, above the x-site or bottom of reach, except for the water sampler until after the samples have been collected (***see Chapter III. WATER COLLECTION PROTOCOLS starting on page 97***). If necessary, a block net can be placed at the bottom of the reach to prevent fish from moving downstream during water collection.

4. Measure the stream at three to five locations, based on variability, to obtain an average width. Multiply the average width by forty to calculate the length of the sample reach.

5. Using two or more 100 meter measuring tapes, lay out the sample reach. When walking the reach, try to stay on or near the bank to minimize fish and habitat disturbance. Remember, minimum reach length is 160 m and maximum is 500 m. If the site has been sampled previously for macroinvertebrates, the original 100 m reach must be included in the fish reach. It is not critical that the lower end of the reach matches the original reach. The fish reach may extend downstream of the macroinvertebrate reach if accessibility/sampleability issues require.

6. Examine the lower and upper ends of the reach to determine if hydrological features (*e.g.,* riffles, plunge pools, etc.) exist to prevent fish passage. If not, place block nets as needed to trap fish within the reach. Be sure the bottom (weighted rope) of the block net is firmly attached to the stream bottom. The net should be upright and the top should be at or above the surface of the stream.

7. Place buckets or plastic tubs on the bank at one or more locations throughout the reach for fish holding. Holding containers (with lids, if necessary, to prohibit fish escape) should be placed in shaded areas if possible to prevent excessive temperatures. Sample jars with formalin may also be placed at these locations for fish preservation.

8. Determine how many and what types of electrofishing equipment will be used. Set the unit voltage according to water conductivity and then shock a small test zone downstream of the reach to evaluate the effectiveness of the unit. Adjust settings according to fish reactions. Further explanation on using the electroshockers can be found in the user's manual. Record the voltage settings on the fish collection form and reset the timer to zero.

9. Before electrofishing begins, ensure that all members of the electrofishing crew are wearing polarized sunglasses, rubber gloves, and appropriate waders for respective stream depth.

10. Begin at the downstream end and electrofish in an upstream pattern going from bank to bank, including all side channels and backwater pools. Thoroughly sample all available habitats and net all fish observed. Keep nets positioned lower in the water in faster current and anywhere turbidity limits fish spotting. Extra attention should be paid to collecting benthic fishes such as darters, sculpins, and catfish. These fish are often missed by crew members holding their nets too high in the water column.

11. Continue working upstream stopping as often as necessary to process fish (***see Field Sample Processing on next page***). Game fish, especially trout, should be measured, photographed, and released regularly to reduce the chance of mortality. Check buckets often to observe fish behavior. If fish are swimming erratically or belly up, it is either time to change the water or process/preserve. Fish that are being retained as vouchers should be placed in formalin jars as necessary while they are alive. Larger fish that can easily be identified in the field may be released after a maximum length for each species is obtained and a photograph is taken. **Be sure that all fish released are counted on the fish collection form. Also, make sure fish are released somewhere downstream of the processing point so that they are not recaptured.**

12. Electrofish to the upper end of the reach, making sure to thoroughly collect around the block net (if used). At this point, process the remaining fish and be sure to record the total shock time on the fish collection form. This is normally a good time to record a rough taxa list from for the stream on the collection form (can be useful later when identifying preserved fish). As the crew is walking back down the stream to the original starting point, they should net any dead fish observed along the way and add them to the specimen collection jars.

13. Review fish collection form (***see Field Data Collection on next page***) to ensure that all necessary information is completed.

Part 2. Field Sample Processing

Field Identification

All fish that can be positively identified in the field will be processed, enumerated, and released if they are in suitable condition (*i.e.,* not dead or dying) except those that are retained for voucher or reference collection reasons (see ***Voucher/Reference Preservation Method below***). Fish that are too large to fit in the sample container should be photographed and released. All RTE (Rare, Threatened, Endangered) and game fish will be released as soon as possible (ideally just after netting and subsequent documentation) to minimize mortality. Released fish will be measured for maximum length of largest specimen and minimum length of smallest specimen for each species. Photograph any specimen if deemed necessary.

Voucher/Reference Preservation Method

At a minimum, at least one fish of each non-RTE species should be vouchered (either preserved in the container or by photograph). It is preferred to voucher at least five individuals of each species. More vouchers are preferred if it is a difficult species to identify or unknown. Minnows, darters, and any other fish that can be difficult to identify should be photographed while still alive and very colorful to help when lab identifications are performed.

Fish retained for voucher or reference collections will be placed in a one gallon Nalgene container approximately 20% (or 1/5th) filled with 37% formalin. Add stream water to the container until it is about half full, and then begin adding fish. Fill the container approximately 70% full with fish. This will reduce bending and distorting of the fish specimens as well as poor preservation. Also, any fish greater than 6” long should have a small incision made in the abdominal wall for proper preservation. Once the jar is full, seal the lid with electrical tape to prevent leakage or spillage. For reference, only jars containing formalin should have a taped lid. Also, make sure the container has an inside and outside label. Fish should remain in formalin for a minimum of two weeks for proper preservation. Normally the fish remain in formalin for several weeks until the end of the field season.

WARNING: Formaldehyde is a known human carcinogen! The vapors and solution may cause severe irritation upon contact with skin and eyes. Use caution when handling and wear nitrile gloves and eye protection. If indoors, always work in a well-ventilated area.

Part 3. Field Data Collection

The following list includes the type of data that will be collected and recorded at each fish community assessment site:

- ✓ Stream name, an-code, and reach length.
- ✓ Date and time of collection.
- ✓ The name and number of each fish species.
- ✓ The minimum and maximum length of each species.
- ✓ DELT (deformity, erosion, lesion, and tumor) anomaly information for any and all fish collected.
- ✓ Photographs of game fish, larger non-game fish, and any RTE (rare, threatened, and endangered) species.
- ✓ Voucher counts. A voucher collection of five or more individuals (if available) of each species (except RTE's) will be retained for later verification.
- ✓ Total shock time, voltage, and the number and type of gear used, the number of netters, and whether block nets were used.

Part 4. Laboratory Documentation or Check-In

Upon return to the office, all samples are to be logged into a Fish Sample Logbook located in the WAB Water laboratory. Each entry is to include: Date of Collection, date received by office, stream name, Random number (if applicable), AN-Code, and collector's initials. If a sample is in multiple jars, each jar is entered individually and designated as "1 of 2" or "2 of 2", as appropriate.

Fish Sampling Quality Assurance/Quality Control

Sample labels are to be accurate and complete and contain all the information discussed above. Sample equipment will be checked, rubbed clean and thoroughly rinsed with stream water before and after each sampling event.

Once a year, all field participants in the WAB attend mandatory training sessions in March-April prior to the initiation of the major sampling season. The purpose of these sessions is to ensure that all field personnel are familiar with sampling protocols and calibrated to sampling standards. A hands-on session concerning the collection and handling of fish samples is included. Any persons unable to attend the annual training session will be instructed and evaluated on the job in the following month by one of the WAB training instructors. In the field, fish sampling teams will consist of three or more people. Individuals who are more experienced in collecting fish will be charged with overseeing the less experienced to assure reinforcement of training and accurate results. This document is also provided to all program personnel for review and use in the field.

Section B. Laboratory Processing of Fish Samples

Materials and Supplies

1. Fume Hood – to vent fumes while processing fish
2. Water – to remove the formaldehyde
3. 20% ethanol – first ethanol wash
4. 50% ethanol – second ethanol wash
5. 70% ethanol – third ethanol wash

Laboratory Safety Precautions

Protective eyewear should be worn during sample processing to prevent contact with the residual formaldehyde or alcohol in the specimens. Formaldehyde is a known carcinogen and alcohol can be a skin irritant and can cause damage to the eyes. All sample processing should occur in a well-ventilated area to reduce inhalation of fumes.

Fish Sample Lab Processing Methods

1. After at least two weeks of preservation in formaldehyde, remove the fish from the container and properly dispose of the waste formaldehyde. Also, mark the date that processing began and your initials in the Fish Sample Logbook. Be sure that the sample information (*e.g.,* date of collection, collector, stream name, county, AN-Code, # of bottle/vial(s), etc.) on the container matches the Fish Sample Logbook.

2. Place the fish back in the container, fill with water, and allow the specimens to soak overnight. Repeat this step three to five times depending on the number of fish in the jar. The more fish specimens that are in the container, the more formalin there is in the fish tissues which takes longer to remove. Add new water each day. The subsequent washings do not need to be disposed of in the same manner as the original waste formalin and may be poured down the drain.

3. For long term preservation, the fish need to be transferred to ethanol. Begin by placing the fish in 20% ethanol and allow them to soak overnight. Then proceed to 50% ethanol (overnight), and finally 70% ethanol for long term. Do not transfer fish directly to 70% ethanol as this will cause hardening, shrinking, and bending of the fish which makes identifications more difficult or impossible.

4. Document the date and your initials in the Fish Sample Logbook when the sample has been fully processed into 70% ethanol.

Fish Laboratory Processing Quality Assurance/Quality Control

Once a year, all field participants in the WAB attend mandatory training sessions in March-April prior to the initiation of the major sampling season. The purpose of these sessions is to ensure that all field personnel are familiar with sampling protocols and calibrated to sampling standards. While a hands-on session concerning the laboratory processing of fish samples is not included, any persons involved with this task will be instructed and evaluated by an experienced, senior biologist before being allowed to conduct this task unsupervised. Individuals who are more experienced in laboratory processing of fish samples will be charged with overseeing the less experienced to assure reinforcement of training and accurate results. This document is also provided to all program personnel for review and use in the lab.

Section C. Identification of Fish

Ultimately, the WAB intends to use fish to bioassess the condition of streams in WV. To accomplish this, the WAB hopes to develop a multi-metric index for assessing fish community data. If developed, the fish MMI/IBI will summarize elements of the structure and function of fish communities. Taxonomic resolution for the fish MMI/IBI will be to the species level.

Materials and Supplies

1. <u>Dissecting microscope</u> - for examination of gross features.
2. <u>Compound microscope</u> - for examining minute features.
3. <u>Fine-tipped forceps</u> - for manipulating specimens.
4. <u>Fine-tipped probes</u> - for manipulating specimens
5. <u>Scalpel</u> – for light dissection of specimens
6. <u>Petri dishes</u> - hold specimens during identification.
7. <u>Alcohol</u> - 70% ethanol is used to preserve the samples and to prevent desiccation during identification.
8. <u>Wash bottle</u> - used for alcohol storage.
9. <u>Fish collection form</u> – add voucher identifications to those done in the field.
10. <u>Fish Measuring Board/Digital Scales</u> – for lab measurements of specimens
11. <u>Taxonomic Keys</u> - (***see List of Taxonomic References on next page***)

List of Taxonomic References

The taxonomic references most frequently used by the WAB biologists for identification of fish include, but are not limited to:

Eddy, S. and J.C. Underhill. 1978. How to Know the Freshwater Fishes. Third Edition. The Pictured Key Nature Series. Wm. C. Brown Co., Dubuque, Iowa.

Jenkins, R.E. and N.M. Burkhead. 1993. Freshwater Fishes of Virginia. American Fisheries Society, Bethesda, Maryland.

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Safety Precautions

Protective eyewear should be worn during sample identification to prevent contact with the residual alcohol in the specimens and debris or at any time while handling alcohol, which can be a skin irritant and can cause damage to the eyes. All sample identification should occur in a well-ventilated area to reduce inhalation of alcohol fumes.

Fish Identification Procedures

Check out the sample in the Fish Sample Logbook by marking the date and your initials. Be sure that the sample information (*e.g.,* date of collection, collector, stream name, county, AN-Code, # of bottle/vial(s), etc.) on the container matches the Fish Sample Logbook. Identify all of the fish in the container and add those records to those identified in the field.

Fish Identification Quality Assurance/Quality Control

The precision of the identification process is evaluated for 2.5% of the samples. These samples are randomly selected after they are received by the laboratory, but before they are sent to the taxonomists for identification. Taxonomists conduct the identification and enumeration of the sample as normal. After they are done, if the sample is designated for a QA/QC check, then all of the specimens are returned to the sample container and passed on to the second taxonomist. The second taxonomist will identify and enumerate the sample in the same fashion as the first. From these two sets of data, two evaluations of precision can be calculated. In addition, voucher and reference specimen identifications will be confirmed by WVDNR fishery biologist expert Dan Cincotta.