CHAPTER 5. BENTHIC MACROINVERTEBRATE COLLECTION PROTOCOLS

Overview

Definitions

MACROINVERTEBRATES - Animals that are large enough to be seen with the naked eye and do not have a backbone.

BENTHIC ORGANISMS (or BENTHOS) - Living organisms that reside on the bottom of streams, rivers, or lakes. Benthos may include vertebrates, invertebrates, or plants.

KICK - One method for collecting benthos. A hand-held net is held in the stream. The stream bed upstream of the net is disturbed using a kicking motion to dislodge the organisms, which then float into the net.

MULTI-METRIC INDEX – An index that incorporates several attributes (community richness, abundance, and tolerance metrics) reflecting biological integrity into one synthetic score.

Benthic Macroinvertebrates as Environmental Indicators

Benthic macroinvertebrates are small animals living among the sediments and stones on the bottom of streams, rivers, and lakes. Insects comprise the largest diversity of these organisms and include mayflies, stoneflies, caddisflies, beetles, midges, crane flies, dragonflies, and others. Other members of the benthic macroinvertebrate community are snails, clams, aquatic worms, and crayfish. These organisms are extremely important in the food chain of aquatic environments as they are important players in the processing and cycling of nutrient and are major food sources for fish and other aquatic animals.

Benthic macroinvertebrates have been used for many years to assess water quality. Currently, they are utilized throughout the world in water quality assessments, as environmental indicators of biological integrity, to describe water quality conditions or health of aquatic ecosystems, and to identify causes of impairment.   Benthic macroinvertebrate communities are known to respond to a wide array of environmental stressors, and in different ways. This response will often make it possible to determine the type of stress that has affected the community. Many macroinvertebrate taxa have relatively long-life cycles. Thus, community structure is a function of past water quality conditions.

Basis of Sampling Method

The sampling methods used in the WVDEP Watershed Assessment Branch (WAB) are qualitative in nature and are adapted from "Rapid Bioassessment Protocols for Use in

Wadeable Rivers and Streams, Second Edition" - U.S. Environmental Protection Agency, July 1999 (EPA 841-B-99-002) (***see Figure 2-27. Cover of USEPA's Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers (Second Edition) in CHAPTER 2. Section C. PAGES 5, 6, 5a, and 6a starting on page 2-65***). Specifically, WAB uses an RBP III-type method, which consists of laboratory processing and identification of field samples (as opposed to RBP I or II methods which involves field identifications of the samples). The RBP III-type method has become the standard for the bioassessment of the benthic macroinvertebrate assemblage and has been adopted and modified for use by many states and organizations conducting such bioassessments.

Selecting Sampling Method and Device

The sampling methods with device are outlined and prioritized below:
RBP III Single Habitat Methods and Devices:

A. Rectangular Dip-Net/Modified Kick-Net – This method is a disturbance-removal sampling type used in riffle/run habitats $\geq$ 0.5-meter-wide and refers to the use of a modified **dip-net/kick-net** or "surber-on-a-stick" type device that has a **rectangular frame** and net opening (0.5 m wide by ~0.3 m tall) with an internal net space that tapers to a point. It is used as a one-man kick-net and requires **four** 0.25 m^2 (0.5 m wide by 0.5 m long in front of the net) **kicks** to get a 1.0 m^2 total kick area. This is the most commonly used method for WAB benthic sampling (> 99% of the time).

> **NOTE:** *This method is comparable with established MMI/IBIs (i.e., WVSCI & GLIMPSS) if you sample a 1.0 m^2 total kick area and have an appropriate flow (i.e., not too high so that water is going over the net, not too fast so that benthos gets pushed out of the net by eddies, and not too little or low so that the benthos does not get pushed into the net).*

B. D-Frame Dip-Net/Kick-Net – This method is a disturbance-removal sampling type used in riffle/run habitats < 0.5-meter-wide and refers to the much smaller, narrower **D-shaped frame** and net opening (1.0 ft. wide by ~0.6 ft. tall) with an internal net space that tapers to a point much like the Rectangular Dip Net Kick-Net does. It should be noted that the length of the net may vary (*i.e.*, some are longer, some are shorter). It takes **eleven** 1.0 ft^2 (1.0 ft. wide by 1.0 ft. long in front of the net) **kicks** to approximate a 1.0 m^2 total kick area. We only use this net when the wetted stream channel is too narrow across to fit the 0.5 m wide Rectangular Dip-Net/Kick-Net, but still has adequate flow to push the benthos into the net. It is very rare to have such conditions in WV, so WAB rarely uses this method.

> **NOTE:** *This method is comparable with established MMIs if you sample a 1.0 m² total kick area and have an appropriate flow (keeping in mind that this device is slightly shorter than the rectangular frame net).*

C. Hand Picking – This method is a disturbance-removal sampling type used in riffle/run habitats in very small or low flow streams where other sampling apparatus cannot be used. It basically involves **hand picking** and washing the substrate into a bucket. It is only used in special surveys or when instructed to get a benthic sample even if it is too low to kick.

> **NOTE:** *This method is not comparable with established MMI/IBIs.*

RBP III Multi-Habitat Methods and Devices:

D. D-Frame Dip-Net/MACS – Also called the Mid-Atlantic Coastal Stream (**MACS**) method, this method is used in the absence of moving water (*i.e.*, in low-gradient streams, glide/pool habitat, or other wetland–like aquatic habitats where flow is insufficient to move suspended materials into a net). The device used is the same **D-shaped frame** and net opening (1.0 ft. wide by ~0.6 ft. tall) as described above. However, the net attached to the frame is different as it does not taper but is flat along the back edge and sampling consists of taking 20 jabs in multiple habitats like submerged vegetation, logs, undercut banks, and snags. It is only used in special surveys or when instructed to get a benthic sample even if no riffle/run habitat is available.

> **NOTE:** *This method is not comparable with established MMI/IBIs.*

It is important to note that the first two methods listed above were established for use by the Watershed Assessment Branch monitoring programs and intended to provide cost-effective techniques with comparable data across the state. The WAB will utilize the Single Habitat Approach whenever possible, using a rectangular dip net (0.5 m wide) or smaller (0.3 m wide) D-net with **a 595-600 μm mesh size** (note that this is different than what is described in the RBP manual) to sample riffle/run habitats. Note that these two are the only sampling protocols that are currently supported by a benthic macroinvertebrate MMI or IBI in WV (***see Section F Special Benthic Sampling Considerations starting on page 5-47***). Special projects outside of the normal Watershed Assessment Branch monitoring agenda (*e.g.*, point source surveys, spills, large river monitoring) may not allow strict adherence to these protocols. These methods are described in detail in the subsequent sections.

Selecting Sampling Sites and Kick Locations

Predominantly, streams in West Virginia are high gradient with coarse substrate materials such as boulder, cobble, and gravel. These physical conditions are responsible for the

typical riffle/run habitats commonly found in most areas of the state. WAB establishes sample sites and assessment reaches on streams based on the best available riffle/run habitat (random sites excluded). There should be at least one square meter of riffle/run habitat in the assessment reach to obtain a complete benthic macroinvertebrate sample.

It is important that the sampling method be selected based on the availability of the reference condition (riffle/run predominant for most of WV) and not of potentially impaired streams. For example, sampling decisions should not be altered for situations where the amount of cobble/gravel substrate in streams influenced by heavy sediment deposition may be substantially reduced from the amount of cobble/gravel substrate expected for the region. That is, sample sites on streams with heavy deposits of fine sediments should not be avoided if it is determined that the sedimentation is not typical of the area and has resulted from poor land-use practices. Occasionally, low gradient streams are encountered that have heavy deposits of fine sediments because of naturally high sedimentation rates. In this case, the Multi-Habitat Approach should be employed. Currently, WAB does not conduct benthic assessments on low gradient streams unless there is a special interest for the resultant data. The decision to sample a stream site is field based and should be made after corroboration by WAB team members or by the most experienced person. In any event, detailed notes describing the situation should be recorded on the field form.

Another concern when locating a benthic sampling site is tributaries or sources that enter the stream within the reach and may significantly alter the water quality. It is extremely important that the benthic data collected always match the water chemistry observed and collected at the X-Site. During the site selection and planning that occurs in the office, every effort is made to try to avoid such situations by locating the site above tributaries and known sources. However, occasionally sources are unknown or moving the site is not possible (*e.g.,* randomly selected sites). The most important thing to do is to always inspect the sample area as thoroughly as possible prior to beginning the benthic collection. Some things to look for are:
1) Significant change in water chemistry (*i.e.,* pH, conductivity, DO, Temperature) from above the source to below the source.
2) Visual indicators that the tributary or source has a significant impact on the mainstem area downstream (*e.g.,* sudden appearance of hydroxides, oils, grease, *etc.* below the tributary or source).
3) In larger streams, pluming of water chemistry along one bank due to an inadequate mixing zone in the mainstem.

In such cases, the entirety of the benthic kicks should be located either above or below the source. Unfortunately, outside of specific directions on the field list, there is little in the way of guidelines on picking one or the other and the samplers must rely on best professional judgment. In the case of a randomly selected site where the X-site is located below a source or tributary with a significant water quality impact to the stream and there is inadequate room to collect benthos in the area below the source, it would be best to treat the source or tributary with significant water chemistry issues using the same rules as sliding the reach downstream around the X-site to avoid crossing stream orders (***see***

CHAPTER 2. Section A. Part 2. Sliding the Reach starting on page 2-9) so that the X-site and benthic collection area are in similar water quality.

Before sampling begins, a 100-meter assessment reach is established containing the X-site (usually located at the downstream terminus of the reach). All assessment activities are conducted within this designated reach including the collection of water samples, benthic macroinvertebrate samples, and habitat assessments. The benthic collector should select kick sampling points with the intent to make collections throughout the entire 100 meters in a diversity of the best available habitats (also called richest-targeted habitat methodology). For example, look for varying conditions within the reach such as fast and slow riffle/runs, deep and shallow riffle/runs, shaded and exposed riffle/runs, and sample from the best available in each observed. In some instances, the best available habitat (*e.g.,* riffle) may be limited to a small area within the reach. In this case, collections should be made within those areas only. However, if riffle areas occur throughout the 100-meter reach, an effort should be made to collect from as many different points within the reach as possible. It is important to sample the diversity of riffle/run conditions if they exist.

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 – Like 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 (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.

Section A. Benthic Macroinvertebrate Sampling

Materials and Reagents

See Figure 5-1 & Figure 5-2 for Diagrams & Picture of most of these materials.

1. <u>Rectangular Frame Dip-Net</u> (AKA Modified Kick-Net) – A net with a 0.5 m wide and 0.3 m high frame with **595-600 µm mesh** openings and 0.5 m nylon bag attached to a four-foot pole will be used to collect benthic macroinvertebrates in riffles and runs.
2. <u>D-Frame Dip Net</u> - A D-frame (D-Net) aquatic dip net with **595-600 µm mesh** openings and 1 ft. nylon bag will be used to sample streams that are too small to be sampled using the rectangular frame dip net.
3. <u>Five-gallon bucket</u> - to composite kick samples in the field.
4. <u># 30 mesh sieve (600 µm)</u> - to remove small particulates and water from samples.
5. <u>Small dish washing scrub brush</u> – aid in removing macroinvertebrates from stream substrate particles such as cobble and cleaning the net.
6. <u>Small plastic container or tray</u> – to temporarily hold the organic materials and elutriate.
7. <u>Gallon-sized sample jars</u> - containers to hold benthic sample and associated debris.
8. <u>Inside and outside labels</u> - for sample identification and tracking.
9. <u>Fine-tipped forceps</u> – for removing organisms from net or sieve.
10. <u>One-liter squirt bottle</u> – for washing benthic organisms from the bucket, sieve, and elutriate container.
11. <u>95% Denatured ethanol</u> - for preservation of benthic macroinvertebrates.
12. <u>Ice chest / cooler</u> - for the storage of samples during transport.
13. <u>Sample log book</u> - for tracking the locations of the biological samples.
14. <u>Scientific collecting permit</u> – Obtained yearly by Watershed Assessment Branch from the WVDNR.

Rectangular Dip-Net/ Modified Kick-Net   D-frame Dip-Net

0.5 m

0.3048 m

0.5 m × 0.5 m = 0.25 m²
0.25 m² × 4 kicks = 1.0 m²

0.3048 m × 0.3048 m = 0.0929 m²
0.0929 m² × 11 kicks = ~1.0 m²

Figure 5-1. Diagram comparing the dimensions and number of kicks necessary to sample 1 m2 of a Rectangular Frame Dip Net versus a D-Frame Dip Net

Figure 5-2. Photo of Materials used in Benthic Macroinvertebrate Sampling

Field Safety Precautions

Rubber gloves and protective eyewear should be worn during sample collection to avoid bacterial contamination and for personal health protection as many streams may have sharp objects embedded in the substrate (*e.g.,* glass, metal, wire, *etc.*). They should also be worn during sample preservation or at any time while handling alcohol, which can be a skin irritant and can cause damage to the eyes.

Part 1. Field Sample Collection Methods

Before any benthic sampling event:

- Fill out a pre-printed sample label 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. Some place the inside label inside the jar before the sample is collected; others do so after the sample is collected. **Just make sure that the inside label gets inside the jar.**
- Fill the sample jar ½ full with 95% denatured ethanol.
- If using a net, check the net to ensure there are no holes or benthic remnants of previous samples. 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.
- Wash the net in the stream to ensure that there are no benthic remnants of previous samples.

Single Habitat-Riffle/Run Habitats using a Rectangular Frame Dip-Net/Kick-Net

✓ ***Comparable for use with current MMI/IBIs***

This method is used in streams having riffle/run habitat and a width ≥ 0.5 meter. This method is to be used even when there is no cobble substrate in the riffle/run area. If the stream has enough flow to wash benthic macroinvertebrates into the net this is the method to use.

Figure 5-3. Photo of Rectangular Frame Dip Net being placed on stream bottom

1. Select a riffle/run area to sample. Position the net on the stream bottom to eliminate gaps under the frame with the net opening upstream. Large rocks or logs that prevent the net from seating properly should be avoided (***see Figure 5-3 on right***).

2. Hold the sampler in position on the substrate while checking for heavy organisms such as clams and snails in an area of about 0.25 m^2 (0.5m wide net x 0.5m upstream) in front of the net. Hand-pick these organisms and place them in the net or the bucket if placed nearby.

> **_IMPORTANT:_**   _**Do not collect large freshwater mussels! Some mussel species are endangered and should not be disturbed.   Record their presence on the field form, photograph and identify them if possible.**_

3. Brush the surfaces of all coarse gravel, cobble, boulder, and bedrock substrate (***see Figure 5-4 on right***). If the substrate is removable, pull it up and hold it underwater in front of the center of the net while brushing all surfaces so that dislodged organisms flow into the net. Cleaned substrate should then be set aside. In low flow situations, these rocks can be placed at the edge of the net in a manner that increases the amount of water flowing through the net. Large substrate that is partially in the kick sample area should only be brushed on that portion which resides in the 0.25 m^2 kick area.

Figure 5-4.   Photograph of the Brushing process in front of the net.

4. Hold the net handle securely while kicking the substrate vigorously for 20 seconds to a depth of 10 cm in an area of about 0.25 m^2 (0.5m wide net x 0.5m upstream) in front of the net (***see Figure 5-5 on right***).   At this time, it may be possible to remove large objects (*e.g.,* cobble, large gravel) from the net while the water is still sweeping through the net.

Figure 5-5. Photograph of the Kicking process in front of the net.

**Figure 5-7. Photographs showing the removal of the
net from the water with an upstream motion.**

5. Remove the net from the water with a quick upstream motion to wash the organisms to the bottom of the net (***see Figure 5-7 above***). Empty the contents of the net into a five-gallon bucket that is partially filled with water (***see Figure 5-6 on right***). Emptying the net after each kick sample is recommended because debris can clog the net mesh causing reduced flow-through and back eddies, both of which can result in the loss of organisms. It is not necessary to fine pick every item from the net at this point, just the get the bulk of the sample into the bucket.

6. Repeat this process until 4 riffle/run habitats have been sampled. This will result in 4 individual kick samples that cover approximately 1 m^2 (4 x 0.25 m^2) of stream substrate. The 4 kick samples will be composited into 1 sample. If a diversity (fast and slow – stacked and flat, *etc.*) of riffle/run types is not present, collect the 4 samples from the best available habitat. It is important to obtain 4 kick samples for the composite. Always record the type and number of each riffle/run sampled on the field assessment form.

Figure 5-6. Photograph of Emptying the net into a 5-Gallon bucket partially filled with stream water.

> **NOTE: The RBP protocol (EPA 841-B-99-002) suggests that 2 square meters of substrate should be sampled and composited at a given site. WAB determined through analysis of duplicate data (2 m^2 versus 1 m^2) and consultation with USEPA Region III biologists that a 1 square meter sample is adequate for characterizing riffle/run streams in West Virginia where the MMI/IBI is to be used for impairment classification.**

7. Inspect the net for clinging organisms. Using a pair of small forceps, remove all the remaining organisms and place them in the bucket.

8. After compositing all four kicks into the bucket, all large objects (rocks, sticks, leaves, *etc.*) should be carefully washed, inspected for organisms, and discarded (***see Figure 5-8 on right***). It is very important to remove as much rough material as possible without losing organisms. This will reduce laboratory sorting time and limit the crushing and grinding that damages benthic specimens. However, if there is an excess of leaves in the sample, this step may become too time intensive to pursue beyond a cursory sorting and removal of the leaves. You can base the amount of time to spend with this by estimating how much longer your partner needs to finish the habitat assessment.

Figure 5-8. Photograph of Biologist inspecting benthic sample and removing rough material (rocks, sticks, and leaves)

9. Elutriate the bucket's soft, organic material (bugs, leaves, CPOM) by using a stirring or swirling motion. Begin pouring some of the elutriated organic material into U.S. Standard 30 sieve. Using a quiet area of the stream or fresh water in the bucket, gently touch the bottom of the sieve to the water surface and rotate it in a circular motion. This will aid in removing fine sediments from the sample. Transfer this material from the sieve into a temporary container (*e.g.,* another bucket, a tray, another sample jar) (***see Figure 5-9 on right***). Repeat this process until almost all the organic material is removed from the bucket. If possible, release any fish and/or salamanders and document the species and number released in the Wildlife Observations section of the Habitat Form. Set the container of elutriated material aside.

Figure 5-9. Photograph of soft, organic material placed and stored in a temporary container.

10. Begin the elutriation process again with the inorganic material (gravel, sand, silt). Pour some of the contents of the bucket through a U.S. Standard 30 sieve. Too much material in the sieve may result in accidental spillage.

11. Using a quiet area of the stream or fresh water in the bucket, gently touch the bottom of the sieve to the water surface and rotate it in a circular motion. This will aid in removing fine sediments from the sample.

> **IMPORTANT:** *Do not immerse the sieve entirely as this will result in the loss of organisms.*

If possible, release all fish and salamanders and document the species and number released in the Wildlife Observations section of the Habitat Form.

12. Pour the hard, inorganic material such as fine gravel and sand from the sieve into a sample jar already 1/2 filled with denatured ethanol (***see Figure 5-10 on right***). Repeat Steps 9-11 until all the inorganic material is sieved and placed into the sample jar. Using a squirt bottle filled with stream water, rinse any remaining material from the bucket onto the sieve.

Figure 5-10. **Photograph of Biologist transferring the hard, inorganic material (*e.g.,* fine gravel, sand, and silt) to a sample jar ½ filled with alcohol.**

13. Use the squirt bottle to aid in removing remnants of the sample from the sieve but avoid getting large amounts of water in the sample jar, as this will dilute the preservative. Inspect the sieve carefully for any remaining organisms and place them in the sample jar.

14. Return to the elutriated soft, organic material (bugs, leaves, CPOM) that was set aside earlier from Step 9. Using a quiet area of the stream or fresh water in the bucket, gently touch the bottom of the sieve to the water surface and rotate it in a circular motion. This will aid in removing fine sediments from the sample. Once all the fine sediments are thoroughly removed, place the elutriated organic contents in the sieve on top of the inorganic material (gravel, sand, silt) previously in the sample jar as in Step 12 (***see Figure 5-11 on right***). Placing the elutriated material on top in the sample jar will protect the often-fragile benthic organisms from damage due to grinding and compaction during transport to the laboratory. Do not invert or shake the sample jar after the elutriated materials are placed inside.

Figure 5-11. **Photograph of Biologist inspecting transferring the soft, organic material (*e.g.,* shredded leaves and benthic organisms) to the sample jar.**

Single Habitat-Riffle/Run Habitat using a D-Frame Dip-Net/Kick-Net

✓ ***Comparable for use with current MMI/IBIs***

In some situations, the stream may be too narrow or shallow to sample using a Rectangular Dip Net. In this case, a D-net will be substituted for sample collection. The methods outlined for the Rectangular Dip Net are applicable when using the D-net in riffle/run streams. The only modification is an increase in the number of kick samples to be collected. This change is necessary to sample approximately the same area (1 square meter). Since the D-net is 1 ft. wide (or $\approx$ 0.3048 m wide), we will sample a square area in front of the net of 0.0929 m^2 (0.333m x 0.333m). To sample 1 m^2, we need to collect from 11 locations (0.0929 m^2 x 11 = 1.0219 m^2).

Hand Picking (Small narrow streams with minimal/interstitial flow)

✓ ***Non-Comparable for use with current MMI/IBIs***

> ***IMPORTANT: This sampling method should only be used for special surveys/projects or if specifically specified in the sampling plan/instructions as it is considered non-comparable to other samples.***

This method should be used in very shallow low-flow situations where there is not enough water to flow over the lip of the Rectangular Dip Net or D-net. Do not collect a sample if there is no interstitial flow in the areas between pools.

1. Sample in areas that would be considered riffles in higher flows. Do not sample in pool habitat. Pick up rocks (small gravel to small boulder) from about 0.25 m^2 (same area as that would be sampled by the Rectangular Dip Net) of substrate. Rub and rinse the rocks into a 5-gallon bucket partially filled with water. Repeat this procedure at four different areas - looking for the best habitats (highest interstitial water flow and most cobble sized rocks).

2. Use the rocks sampled to complete the benthic substrate section of the Habitat Assessment Form.

3. Pour the entire contents of the bucket through a U.S. Standard 30 sieve. Using a squirt bottle, rinse any remaining organisms from the bucket onto the sieve. Using forceps, remove any remaining organisms and transfer to jar. Place sample jar in cooler or other air-tight container designated for benthic macroinvertebrates.

MACS (Multi-habitat Approach for Low Gradient Streams, Glide/Pool Habitat)

✓ ***D-Frame Dip-Net=Non-Comparable for use with current MMI/IBIs***

The RBP procedures described above are only applicable to flowing, wadeable streams. The Multi-Habitat Approach is based on protocols developed by the Mid-Atlantic Coastal Streams (MACS) Workgroup, which are employed in low gradient, slow moving streams.

> ***IMPORTANT:*** ***This method is to be used only in wetland type habitat where flow is insufficient to move suspended materials into a net. This type of sampling is considered non-comparable. Therefore, it should only be used for special surveys/projects or if specifically specified in the sampling plan/instructions.***

1. Determine the types of productive habitat to be sampled and the percentage of each habitat within the sample station. Productive habitats are snags, vegetated banks, and submerged macrophytes. A total of 20 jab-sweeps (see next step) are collected based on the proportion of productive habitats available in the 100-meter assessment area. For example, if 50% of the habitat is snag material and 50% is submerged macrophytes, then 10 jab-sweeps (50%) are taken in snags and 10 jab-sweeps (50%) are taken in submerged macrophytes. If a habitat type is rare (<5%), it is not sampled.

2. Collect macroinvertebrates by jab-sweeping the net into productive and stable habitat. A "jab-sweep" is an aggressive thrusting and sweeping of the net into productive habitat for one half meter. **Make only one jab-sweep; resist the urge to re-sweep!** A total of 20 jab-sweeps will be combined to complete the sample. The precise jab-sweep technique will vary with the type of habitat being sampled.

   A. **Snags** –Disturb the snag area first by kicking it to dislodge the organisms. Then quickly jab-sweep the net into small sticks and branches or scrape the net along the lower surface of logs. Medium sized snag material is best – sticks and branches. Large logs should be avoided because they are generally difficult to sample adequately.

   B. **Submerged Macrophytes** - In deep water, drag the net through the vegetation from the bottom to the water surface (maximum of 0.5 m each jab). In shallow water, bump the net along the stream bottom within the macrophyte bed, avoiding sediments where possible.

   C. **Vegetated and Undercut Banks** - Use the snag collection method for collecting from roots and emergent plants that are on the lower banks of streams. Submerged areas of undercut banks are included here. Sample unvegetated banks by bumping the net along the substrate.

3. After five jab-sweeps have been collected, empty the net into a 5-gallon bucket containing stream water. (The net may be emptied more frequently, depending on

the amount of material.) Repeat until 20 jab-sweeps have been collected.

The remaining procedure is the same as for the Rectangular Dip Net. Follow steps 8 through 14 under Sample Collection Methods – I. Rectangular Dip Net (Riffle/Run Habitats = Comparable) to complete field processing and preservation.

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Part 2. Field Sample Preservation Methods

1. Fill a gallon sized sample jar about 75% full with 95% denatured ethanol. The goal is to reach a concentration of ethanol near 70% after the sample and some water has been added. If there is a small amount of water and organic material in the sample, it may not be necessary to fill the jar to 75% capacity to reach a 70% concentration. It is important that sufficient ethanol be used to reach 70% concentration. In addition, enough alcohol should be added to at least immerse all the material in the jar. If more ethanol is needed, it can be added after the sample is received at the laboratory.

2. Make sure that there is a waterproof label filled out with pencil inside the jar and a label affixed to the outside of the jar using clear packing tape. Include stream name, AN-Code, and date on both labels. Place the jar in a cooler or other container designated for the storage and transport of benthic macroinvertebrate samples to the laboratory.

3. Avoid agitating the sample jars as much as possible. Do not invert the jars.

Part 3. Laboratory Documentation or Check-In

Upon return to the office, all samples are to be logged into a Benthic Macroinvertebrate Sample Logbook. 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.

Benthic 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 for residual benthic material, rubbed clean and thoroughly rinsed with stream water before and after each sampling event.

Duplicate samples will be collected from 2.5% of the sites sampled. Most these events will occur when there are at least two people on the sampling team (which is the norm). However, a new effort will be made to have duplicate samples conducted by one sampler to measure within-sampler variance in the collection methodology. Such single-sampler

duplicates can occur whenever a sampler is working alone or in a team and the opportunity (both in time and available comparable habitat) is available to collect two sets of samples. In such cases, only the Benthic Collection Information portion of the field form must be filled out as one cannot have a within-sampler RBP habitat duplicate.

Benthic macroinvertebrates will be collected along with other activities at the designated duplicate WAB sites. Both duplicates are collected at the same date and approximate time (as equipment sharing will allow) by different individuals. Extreme care is taken to ensure that the second duplicate is not taken from an area that may have been depleted by the first duplicate. The duplicate data will be analyzed to ensure precision and repeatability of the sampling technique. Every effort is made to ensure that different teams perform the duplicate sampling throughout the sampling season to ensure that all variability is being captured. The variances between individual techniques will be documented and used in future training sessions or individual re-training. In addition, the duplicate data is looked at by Watershed Assessment Branch staff and scrutinized to find any possible discrepancies, contamination, or faults in the sampling methods and techniques. Any problems are brought to the attention of the program management and steps are made to immediately correct the problem. Data that is related to the problem are flagged with notes concerning the details of the situation so that decisions can be made whether to include the data in any further assessments or analysis. *See CHAPTER 14. Section A. Blanks and Duplicates starting on page 14-1 for additional information.*

Once a year, all field participants in the WAB attend mandatory training sessions. 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 benthic macroinvertebrate samples is included. In the field, biological sampling teams will consist of two people. Individuals who are more experienced in collecting benthic macroinvertebrates will be teamed up with the less experienced to ensure 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 Benthic Macroinvertebrate Samples

Benthic macroinvertebrate sample sorting is performed utilizing a modification of U.S EPA's RBP 200-count sub-sampling method. It is described in more detail in subsequent sections.

Sorting macroinvertebrates (a procedure often referred to as "bug picking") is an extremely important step in the biological research performed by the Watershed Assessment Branch. The quality of the work performed by the "picker" influences the quality of subsequent processes, such as identification and data analysis. A competent "picker" must be able to recognize the morphological diversity of aquatic organisms, as well as the various methods these organisms may use to hide themselves from predators. The outcome of the final study may be affected if only a few organisms are overlooked

during the picking process.

The biologists in the Watershed Assessment Branch acknowledge the fact that the sorting process can be tedious at times. The picker is advised to discuss alternate sorting techniques that may be applied to difficult samples with senior biologists. All types of aquatic macroinvertebrates should be picked including insects, snails, clams, crustaceans (including crayfish), and worms.

Materials and Supplies

1. <u>Sample jar</u> - contains the unprocessed sample.
2. <u>Sample vial</u> - for storage of processed sample.
3. <u>Enamel pans</u> - contains sample during the sorting process.
4. <u>Denatured ethanol</u> - preservative used in unprocessed and processed samples.
5. <u># 30 sieve</u> - used to separate alcohol and fine debris from the sample prior to picking.
6. <u>Gridded sorting tray</u> – (***See Figure 5-12 on right***) a Plexiglas framed sorting tray is used to evenly distribute the washed sample and for randomly selecting the 200-organism

Figure 5-12. Photograph of a Home-Made Gridded Sorting Tray featuring a random number matrix on the bottom.

subsample. The internal dimension of the tray is 40 inches by 10 inches. There are 100 grids in the tray and each is 2 inches by 2 inches in dimension.
7. <u>Cookie cutter</u> - a homemade cookie cutter, 2 inches by 2 inches is used in conjunction with the sorting tray to isolate each of the subsamples.
8. <u>Labels</u> - Self-adhesive labels are used to identify the contents of the sample bottle (*i.e.,* the picked sample).
9. <u>Tape</u> - used on label as additional adhesive.
10. <u>Pencil</u> - used to label sample bottle.
11. <u>Crucible</u> - or other small container, is used for short term, intermediate storage of the sample during the picking process.
12. <u>Forceps</u> - Fine tipped forceps are used to remove the organisms from the debris.
13. <u>Illuminated magnifier</u> - an optical aid to illuminate and magnify the sample during the picking process. Alternatively, magnifying visors and a desk lamp can be used.
14. <u>Squirt bottle </u>- filled with alcohol, used to rinse organisms into sample bottle.
15. <u>Plexiglas</u> - used to cover sample overnight to prevent evaporation.
16. <u>Counter </u>– used to count the number of organism removed from the sample.

Laboratory Safety Precautions

Protective eyewear should be worn during sample processing 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 processing should occur in a well-ventilated area to reduce inhalation of alcohol fumes.

Benthic Sample Processing Methods

1. Select the sample to be sorted. A supervising biologist may provide the picker with a specific sample to be sorted, based on priority. Be sure that the sample information (*e.g.,* date of collection, collector, stream name, county, AN-Code, *etc.*) on the vial matches the Benthic Macroinvertebrate Sample Logbook. Also, mark the sign-out date for processing and your initials in the logbook.

2. Select a small bottle/vial that will hold the organisms after sorting is completed. Usually 10 mL bottle or 4-dram Vial is adequate for a 200-organisms sub-sample. A larger bottle or vial may be needed if the sample contains large organisms such as crayfish. In some cases, it may be necessary to split the sample into multiple bottles or vials.

3. Prepare a label for the sample bottle/vial(s):
    - It may be necessary to prepare a second label for the outside of the bottle/vial. If so, avoid using self-adhesive labels as the adhesive tends to lose its stickiness after exposure to alcohol.
    - Use a pencil or an archival quality ink pen on the labels (*e.g.,* Pigma Pens). Most inks will run if alcohol is spilled on the label.
    - Be sure to copy all information on the sample jar label onto the self-adhesive label. The label must include the following information:
        - ✓ Stream Name
        - ✓ Station Number (Random Number and/or AN-Code)
        - ✓ Sample Date
        - ✓ County
        - ✓ Collection Method
        - ✓ Initials of Sample Collector
        - ✓ Initials of Sample Processor
        - ✓ # of grids picked (to be added after the sample picking is done)
        - ✓ # of organisms in final sample (to be added after the sample picking is done)
        - ✓ Vial # out of Total Vials (to be added after the sample picking is done)
      If any of this information is missing from the original sample jar label, notify the supervising biologist so that the error can be corrected.

4. Prepare the sample for sorting. This step is performed in a sink and should be done under a fume hood or in a well-ventilated area.

a. Under a fume hood, open sample jar and pour contents into the **# 30 (600 μm) mesh** sieve. Capture the ethanol and transfer it to a long-term holding container for later disposal.

b. Rinse sample jar into sieve with water and examine jar to make sure all detritus has been removed.

c. Rinse the contents of the sieve in tap water to remove remaining alcohol and to rinse out fine sand and sediment.

d. Carefully rinse any large detritus (*i.e.* leaves) or stones, making sure that all organisms on these items are returned to the sieve. Discard the leaves and rocks after rinsing.

e. Place the contents of the sieve in the gridded sorting tray. Fill the tray 1/3 full with water and gently swirl it until the contents are evenly distributed (*See Figure 5-13 on right*). If the sample was divided into more than one jar, wash the contents of the additional sample jars and combine them with the first jar's contents in the sorting tray at this point.

f. Using a random number generator, select the first grid to be picked (*see Figure 5-14 on right*). Using the "cookie cutter", isolate the organisms within the chosen grid and scoop the contents of the grid into a white enamel
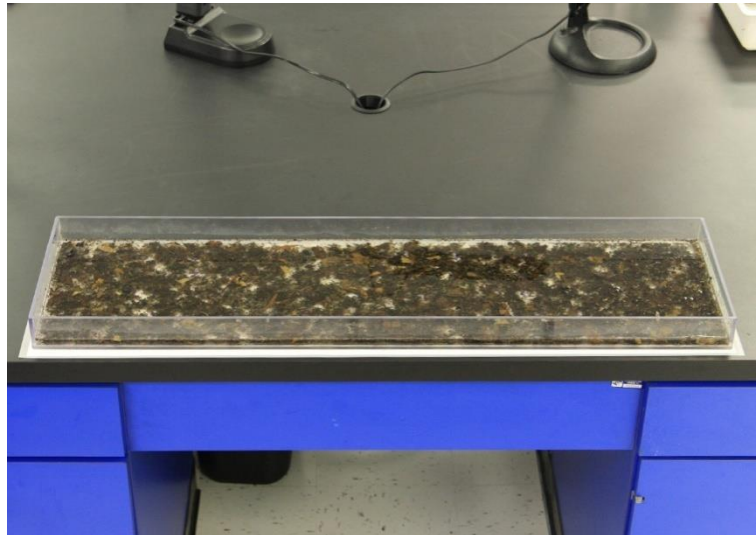
Figure 5-13. Photograph of a Gridded Sorting Tray with sample contents evenly distributed in water.
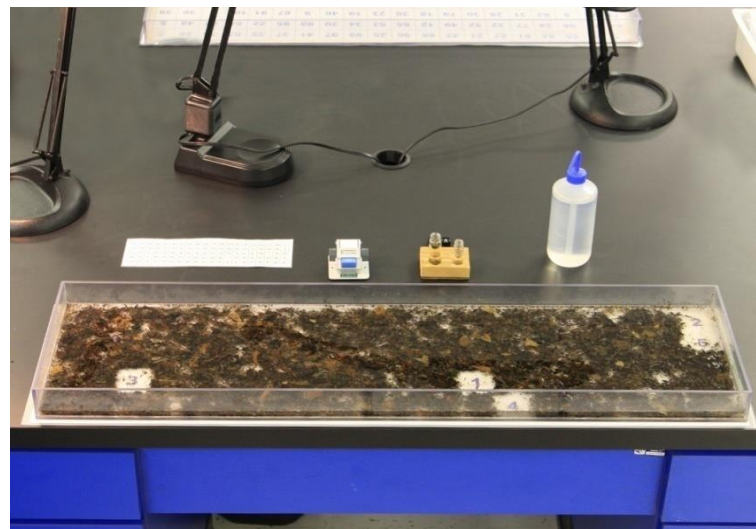
Figure 5-14. Photograph of a Gridded Sorting Tray with 5 grids randomly removed.

Note that the sequence of numbers on the bottom of the tray known by referencing a piece of paper that has the locations of each grid mapped out.

pan with just enough water in the bottom to easily maneuver the organisms. Be careful not to destroy any organisms during this step. Organisms with their head inside the grid are to be included within the grid. If you can't distinguish which end is the head, then the organism belongs in the grid that contains the largest portion of the body.

5. Sorting (Picking)

    a. Fill a crucible or temporary storage vial with 75% ethanol. If preferred, another small wide-mouth container may be substituted for the crucible.
        Note: A small piece of tape, rolled into a ring so the adhesive is exposed, may be attached to the bottom of the crucible to prevent tipping.

    b. Using fine-tipped forceps and illuminated magnifier or magni-visor (*see Figure 5-15 on right*), remove all invertebrates from the

Figure 5-15. Photograph of Biologist sorting a benthic sample under an illuminated magnifier. Note the enamel pan filled with some water and the temporary sample container.

sub-sample and transfer to the alcohol filled crucible or labeled storage vial. Keep track of the number of organisms that have been picked.

    c. If leaves are present, be sure to examine both surfaces. Examine the debris for unusual clumps of twigs, leaves, or sand, which may be protective cases for some organisms. If cases are found, both the case and the organism should be picked. If the organism is in the case, the case and organism should be kept together. If an empty case is found, it should also be removed, but not counted towards the final number of organisms picked.

    d. If there is any doubt to the identity of an object (is it a seed or a bug?), it should be picked, but not counted. A senior biologist should be notified if many questionable objects are present.

    e. When all the organisms appear to have been removed from the pan, agitate the contents of the pan and look again. Often the agitation will reorient an organism that was previously overlooked.

    f. Have a senior biologist inspect the pan after picking has been completed. The biologist will point out any organisms that have been overlooked or misidentified as detritus. As the picker becomes more proficient at his/her task, this step will be reduced in frequency.

g. Discard the contents of the enamel pan by pouring the contents through a "waste sieve" in the sink. The contents of the waste sieve may be emptied into the trash as necessary.

h. Continue the Sorting process repeatedly (steps 4-f through 5-e) until a subsample of 200 (+/- 20% is reached) (**see Figure 5-14 on page 5-18**). Several rules must be observed to get a subsample that is both random and representative of the whole sample.

1. The total organisms in the sample must be between 160 and 240 organisms. If fewer than 160 organisms have been collected, another grid is randomly chosen and steps 4-f through 5-e are repeated until at least 160 organisms are obtained or until the entire sample has been picked. Every attempt should be made to get the final subsample as close to 200 as possible. Therefore, the person conducting the sub-sampling should keep track of the approximate number or organisms per grid to know if one more grid will get the subsample number as close to 200 as possible.
2. If subsampling should result in **more than 240 organisms** in the subsample, then the subsample should be re-subsampled to bring the number of organisms down to the 200 (+/- 20%) organism goal.
3. Should the 200 (+/- 20%) organism goal be reached in less than 4 grids, then picking should continue until 4 total grids have been picked and then that subsample should be re-subsampled to reach the 200 (+/- 20%) organism goal. This step will ensure representativeness of the subsample compared to the total sample.

> **NOTE: For further information about subsampling rules, refer to the *USEPA Rapid Bioassessment Protocol References listed in CHAPTER 2. Section C. Part 1. PAGES 5, 6, 5a, and 6a USEPA's Rapid Bioassessment Protocol Visual-Based Habitat Assessment starting on page 2-65.***

> **NOTE: Based on WVDEP's experience, >90% of the time, 4 or more grids out of 100 will need to be picked to reach the target 200 organism subsample for a 1m² kick area.**

i. Place the label made earlier inside the bottle/vial(s). If a second label is prepared for the outside of the bottle/vial, then affix it using tape. Be sure to write down the # of grids picked, # of organisms in final subsample, and if applicable, the Bottle/Vial # out of the Total Bottles/Vials for the subsample before you put the label inside the bottle/vial(s).

j. Pour the subsample contents of the crucible (or temporary container) into the final storage bottle/vial(s). Use a squirt bottle containing alcohol to rinse the organisms from the crucible. Make sure that all organisms in the bottle/vial are

fully submerged in the alcohol and that none are clinging to the sides of the bottle. Use the squirt bottle to rinse the sides of the bottle/vial, if necessary.

k. If required, return the remainder of the unpicked sample to the original sample jar and preserve with alcohol. These samples may be processed later to determine picking efficiency.

l. After a sample has been picked, record the date or return and your initials in the Benthic Macroinvertebrate Sample Logbook to indicate that the sample was returned from processing. Be sure that the sample information (*e.g.,* date of collection, collector, stream name, county, AN-Code, # of bottle/vial(s), *etc.*) on the bottle/vial(s) matches the Benthic Macroinvertebrate Sample Logbook.

Benthic Laboratory Processing Quality Assurance/Quality Control

Sorting efficiency is evaluated for 5% of the samples. These samples are randomly selected after they are received by the laboratory, but before they are sent to the pickers. Pickers conduct processing of the sample as normal, but each time they are done picking a subsample grid in the enamel pan, a second picker (usually a senior biologist) will review the pan for any missed organisms. The missed organisms for the entire sample are totaled.

Percent Sorting Efficiency (PSE)

The Percent Sorting Efficiency (PSE) (AKA Bias) can then be calculated by the following formula:

Equation 2. Percent Sorting Efficiency (PSE)

$$\frac{\text{\# Organisms Originally Sorted}}{\text{\# Organisms Recovered by Checker} + \text{\# Organisms Originally Sorted}} \; X \; 100 = PSE$$

A PSE >= 90% is considered passing.

Pickers may also be instructed to retain the unpicked portion. The unpicked portion can then be checked by a senior biologist to determine if the number of grids that need to be picked to get a second subsample is comparable to the original pick. This will indicate if the sample was evenly distributed in the tray.

Section C. Identification of Benthic Macroinvertebrates

Ultimately, the WAB uses benthic macroinvertebrates to bioassess the condition of wadeable streams in WV. To accomplish this, the WAB has multiple tools available (*see Section E. Benthic Macroinvertebrate Data Analysis starting on page 5-38*), each with unique taxonomic resolution requirements (*e.g.,* Family vs. Genus level taxonomy) that must be considered during the identification process. Ideally, all aquatic macroinvertebrate taxa should be identified to the genus level or lowest practical taxon

including insects, snails, clams, crustaceans (including crayfish), and worms.

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>Petri dishes</u> - hold specimens during identification.
6. <u>Alcohol</u> - 75% ethanol is used to preserve the samples and to prevent desiccation during identification.
7. <u>Wash bottle</u> - used for alcohol storage.
8. <u>Microscope slides, cover slips, and mounting media</u> - for examination of tiny specimens and/or body parts under a compound microscope.
9. <u>Benthic macroinvertebrate lab sheet</u> - standard for recording results of identification and enumeration (***see Figure 5-16 on page 5-31***).
10. <u>Taxonomic Keys</u> - (***see List of Taxonomic References below***).

List of Taxonomic References, Lists, and Photo Guides

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

General Keys

Brigham, A.R., W.U. Brigham, and A. Gnilka (eds.). 1982. Aquatic Insects and Oligochaetes North and South Carolina. Midwest Aquatic Enterprises, Mahomet, IL.

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Pennack, R.W. 1978. Fresh-water Invertebrates of the United States. 2nd edition. John Wiley & Sons, New York.

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Annelida

Brinkhurst, R.O. 1986. Guide to the freshwater aquatic microdile oligochaetes of North America. *Canadian Special Publication of Fisheries and Aquatic Sciences* **84**: 259 pp.

Klemm, D.J. 1973. Biota of Freshwater Ecosystems, Identification Manual No. 8. Freshwater Leeches (Annelida: Hirudinea) of North America. EPA-905-R-72-117. WPCRS No. 18050, ELD04/72. Supt. Doc. No. 5501-0391, United States Environmental Protection Agency, Washington, D.C. 53 pp. Available on-line at: *http://www.epa.gov/nscep/index.html*

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Klemm, D.J. (ed.). 1985. A guide to the freshwater Annelida (Polychaeta, naidid and tubificid Oligochaeta, and Hirudinea) of North America. Kendall/Hunt Publishing Co., Dubuque, Iowa.

Klemm, D.J. 1995. Identification Guide to the Freshwater Leeches (Annelida: Hirudinea) of Florida and Other Southern States. Florida Department of Environmental Protection, Division of Water Facilities, Tallahassee, FL. Available on-line at: *http://publicfiles.dep.state.fl.us/dear/labs/biology/biokeys/leeches.pdf*

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Stimpson, K.S., D.J. Klemm, and J.K. Hiltunen. 1982. A Guide to the Freshwater Tubificidae (Annelida: Clitellata: Oligochaeta) of North America. EPA-600-3-82-033. United States Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio. Available on-line at: *http://www.epa.gov/nscep/index.html*

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Hobbs, H.H., Jr. 1972. Biota of Freshwater Ecosystems, Identification Manual No. 9. Crayfishes (Astacidae) of North and Middle America. EPA-905-R-72-116. WPCRS No. 18050, ELD05/72. Supt. Doc. No. 5501-0399, United States Environmental Protection Agency, Washington, D.C. 173 pp. Available on-line at: *http://www.epa.gov/nscep/index.html*

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Jezerinac, R.F., G.W. Stocker, and D.C. Tarter. 1995. The Crayfishes (Decapoda: Cambaridae) of West Virginia. Ohio Biological Survey Bulletin. New Series. Vol. 10, No.1.

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https://files.nc.gov/ncdeq/Water%20Quality/Environmental%20Sciences/BAU/Benthos%20Reference/BAU%20Taxonomy%20-%20Ephemeroptera%20-%2021Oct13%20Full%20version.pdf

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Berner, L. and M.L. Pescador. 1988. 2nd Ed. The mayflies of Florida. Univ. Florida Press, Gainesville, Florida. 352 pp.

Burks, B.D. 1953. The mayflies, or Ephemeroptera, of Illinois. Illinois Natural History Survey (Urbana) Bulletin 26, Part 1:1-211.

Edmunds, G.F., Jr., S.L. Jensen, and L. Berner. 1976. Mayflies of North and Central America. University of Minnesota Press.

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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.

Macroinvertebrate Identification Procedures

1. Check out the sample in the Benthic Macroinvertebrate Sample Logbook. The laboratory manager may pre-assign which taxonomist gets which sample and if that sample will be subject to a QA check. Be sure that the sample information (*e.g.,* date of collection, collector, stream name, county, AN-Code, # of bottle/vial(s), *etc.*) on the vial matches the Benthic Macroinvertebrate Sample Logbook. Also, mark the sign-out date for identification and your initials in the logbook.

2. Complete the top portion of a "Benthic Macroinvertebrate Lab Sheet" with the sample information (*e.g.,* date of collection, collector, stream name, county, AN-Code, *etc.*) (***see Figure 5-16 on page 5-31***).

3. Using the taxonomic keys listed above (***see List of Taxonomic References starting on page 5-22***); identify the contents of the sample to the family or genus level, depending on the specifications of the project. Use the reference collection as additional confirmation, if necessary. **IF YOU HAVE ANY UNCERTAINTY ABOUT THE IDENTIFICATION OF A SPECIMEN, CONSULT A FELLOW BIOLOGIST FOR CONFIRMATION.** If an organism is too small or damaged and cannot be identified to the designated taxonomic level, identify it to the lowest positively-identified taxon and document why the identification was not complete (*e.g.,* immature or damaged specimens).

4. Record results of the identification and enumeration on a "Benthic Macroinvertebrate Lab Sheet" (***see Figure 5-16 on the next page***). Be sure to include notes for each taxon about immature or damaged specimens, life stages other than larvae (*i.e.,* Adults and Pupae), terrestrial specimens that were picked inadvertently, numbers of

specimens pulled for reference collections, and likely characters that would place the specimen in a lower level taxon if you are unfamiliar with the organism.

5. Return the specimens to the original sample bottle and mark the label with an "X" to indicate the sample has been identified.

6. Return the identified sample bottle/vial(s) and corresponding lab sheet. Be sure that the sample information (*e.g.,* date of collection, collector, stream name, county, AN-Code, # of bottle/vial(s), *etc.*) on the vial matches the Benthic Macroinvertebrate Sample Logbook. Also, mark the date of return from identification and your initials in the logbook.

WVDEP-WAB BENTHIC MACROINVERTEBRATE LAB SHEET

Stream Name: _____ AN-Code: WV_____ R#: _____

Sample ID: _____ Collection Date (mm/dd/yy): _____ County, State: _____

Sorted by: _____ Number of Grids Picked: _____ Number of Organisms Picked: _____

ID By: _____   Collected By: _____

Taxon ID/Taxon	Count	Taxon ID/Taxon	Count	Taxon ID/Taxon	Count
Annelida		**Plecoptera**		**Diptera (Chronomidae)**	
Amphipoda					
Isopoda					
				Diptera (other)	
Decapoda		**Trichoptera**			
Ephemeroptera					
		Megaloptera			
				Mollusca	
Odonata					
		Coleoptera			
				Other Taxa	

Figure 5-16. Example of a Benthic Macroinvertebrate Lab Sheet.

Benthic Laboratory Identification Quality Assurance/Quality Control

Part 1. SFS Taxonomic Certification Program

Program Background and Justification

The accurate and precise identification and classification of organisms provides the foundation for many ecological investigations of streams, rivers, and lakes, including biological assessment and monitoring programs aimed at evaluating the quality of habitat and water.

High quality taxonomy is crucial to credible ecological studies and reliable bioassessment programs. However, there is concern that:

1) There are many errors and inaccuracies associated with the taxonomy of some on-going programs;
2) There is no recognized protocol in North America for evaluating the taxonomic ability of people identifying invertebrates; and;
3) Academic support for faculty positions and student training related to non-molecular, organismal taxonomy is declining.

These concerns have been expressed by many state and federal agencies (*e.g.*, Kentucky, North Carolina; Environment Canada, US Environmental Protection Agency, US Geological Survey) involved in environmental regulation and monitoring as well as by individuals involved in both basic and applied scientific research.

Consequently, the Society for Freshwater Science (SFS, formerly North American Benthological Society or NABS) decided to implement a certification program for those involved with macroinvertebrate identification in North America. This program will certify that trained and skilled persons are providing aquatic invertebrate identifications. In addition, it is anticipated that this program will help promote undergraduate and graduate training of new taxonomic experts, the training of taxonomic technicians through workshops, the development of new manuals for identifying aquatic invertebrates, and taxonomic excellence in other disciplines and other parts of the world. The certification program will test a candidate's knowledge and skills in aquatic invertebrate taxonomy and will provide the successful applicant with a **certificate of proficiency that lasts five years**.

Genus level testing is conducted in one of two ways:
1) Specimen-based using actual whole or slide mounted specimens provided by SFS or
2) Online image-based (like the Family level test for Aquatic Insects).

In addition, Genus level testing is broken out into multiple taxonomic groups and by US geographic region (*i.e.*, Eastern or *occurring east of the Rocky Mountains* vs. Western or

occurring in and west of the Rocky Mountains). The five Taxonomic Groups (eastern and western) are as follows:

1. Ephemeroptera, Plecoptera, and Trichoptera (EPT) genera (nymphs and larvae only) *(Image-Based test)*,
2. Chironomidae genera (larvae only) *(Specimen-Based test)*,
3. Genera of Crustacea and arthropods (including insects) other than EPT and Chironomidae (immatures and adults as appropriate) *(Specimen-Based test)*,
4. Oligochaeta genera *(Specimen-Based test)*,
5. Mollusca genera (field identifications are not included) *(Specimen-Based test)*.

The WVDEP headquarters in Kanawha City is a certified testing center for all taxonomic identification tests offered by the SFS Taxonomic Certification Program (TCP). Individuals may be certified at either the Family or Genus-level. Details about testing can be found online at:

> *http://www.dep.wv.gov/WWE/watershed/bio_fish/Documents/TaxonomicIdentificationCertification.docx*

Part 2. Benthic Identification QA/QC Metrics

The precision of the identification process is evaluated for at least 5% of the samples. The samples are randomly selected after they are received by the laboratory and picked, but before they are sent to the taxonomists. A proper sample for identification QA/QC must have a total count that falls within the 200 +/- 20% subsample size (*i.e.,* samples with counts below 160 should not be selected). 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 the specimens (mounted or loose) are 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, several evaluations of precision can be calculated:

Percent Difference in Enumeration (PDE)

The Percent Difference in Enumeration (PDE) is calculated by the following formula:

Equation 3. Percent Difference in Enumeration (PDE)

$$\frac{(n_1 - n_2)}{(n_1 + n_2)} \times 100 = PDE$$

Where:

n_1 = # of organisms counted by taxonomist 1

n_2 = # of organisms counted by taxonomist 2

A PDE <=10% is considered passing.

Percent Taxonomic Difference (PTD)

Percent Taxonomic Difference is a comparison of the accuracy in identifications from one taxonomist to another. This begins using a Taxonomic Comparison Form. On this form, the identifications by both taxonomists are matched up to each other and then difference in enumerations between the two taxonomists is compared. The number of agreements is defined as the lower of the two numbers for the given taxon being compared.

The Percent Taxonomic Difference (PTD) is calculated by the following formula:

Equation 4. Percent Taxonomic Difference (PTD)

$$\left[1 - \frac{(\mathbf{comp_{pos}})}{(\mathbf{N})} \right] \times 100 = \mathbf{PTD}$$

Where:
   N = Highest count of organisms from taxonomist 1 or 2
   $comp_{pos}$ = Total # of taxonomic agreements from the Taxonomic Comparison Form

A PTD <=10% is considered passing for Family Level taxonomy.
A PTD <=15% is considered passing for Genus Level taxonomy.

PTD is not an evaluation of which taxonomist is correct. However, the process does include a method by which conflicts in taxonomic identification are reconciled. After the PTD is calculated, both taxonomists and a third party sit down and attempt to ascertain where the differences in identifications and enumerations are coming from. Reasons for the differences include:

1. **Misidentification of the Taxon**.
      Example 1. One of the taxonomists may not be as familiar with a taxon as the other and keyed it wrong. This may be a consistent error in all the QA samples involving the taxonomists.
      Example 2. One taxonomist is using an outdated key that refers to a taxon that has been lumped with or is synonymous with another taxon.
      Example 3. One of the taxonomists accidentally included a terrestrial specimen from a taxon that is very similar to an aquatic taxon.

2. **Taxonomic Resolution**.
      Example 1. The first taxonomist may have inadvertently damaged a key feature of a specimen that prevented it from being identified by the second taxonomist to the same taxonomic level.
      Example 2. One of the taxonomists may be better experienced and familiar with that a given taxon and can identify it the lower taxonomic level where the other taxonomist cannot.

3. **Specimens Lost Between Taxonomists**. This should be kept to a minimum if the two taxonomists view the sample before it is put back into the bottle/vial(s).

   Example 1. Specimens may have been pulled from the sample (*e.g.,* Reference Collection or Slide Mounting) and not viewed by the second taxonomist.

   Example 2. Specimens stuck to the bodies of larger organisms (*e.g.,* an Elmidae beetle stuck in the "armpit" of a large *Corydalus* specimen) are missed by one taxonomist.

   Example 3. One taxonomist was including pupae, body parts, or empty shells/cases in the count while the other was not.

   Example 4. One taxonomist may have counted partial organisms as whole organisms. This is most common with Oligochaeta as the head are difficult to find and they often get broken up into pieces easily.

4. **Transcription, Translation, and Typographic (TTT) Errors**.

   Example 1. One taxonomist meant to write down an 11 and accidentally wrote down a 1.

   Example 2. The person who calculated the PTD mistook an 11 for a 2.

   Example 3. The taxonomist wrote down a very similarly spelled taxon (*e.g., Thienemannimyia* vs. *Thienemanniella* vs. *Thienemannia*).

   Example 5. After this reconciliation, the PTD can be recalculated correcting for these most of these errors (called a corrected PTD).

Section D. Benthic Macroinvertebrate Sample Comparability

Before a sample can go thru data analysis via an IBI or MMI, it must be checked for index comparability. Hopefully, all directions presented in the sections above followed. However, this is not always the case. Sometimes errors or mistakes are made when the sample is collected (*e.g.,* the sampler did not know that the stream was dry the week before), processed (*e.g.,* not enough or too many specimens were picked), or identified (*e.g.,* the identifier got the counts reversed between two taxa). Other times, the sample is not comparable due to restrictions of the index. And in some rare instances, the sample is collected despite known problems with comparability (*e.g.,* Limestone dominated stream, non-comparable method or equipment, after a scour event, etc.) because the goals of the project dictate the need to sample in those conditions (*e.g.,* scour recovery survey) or to get any sort of benthic macroinvertebrate sample (*e.g.,* spill response survey). The following flow chart (**Figure 5-17 on next page**) is designed to help decide about the comparability of the sample in the context of the WVSCI and GLIMPSS MMI/IBIs.
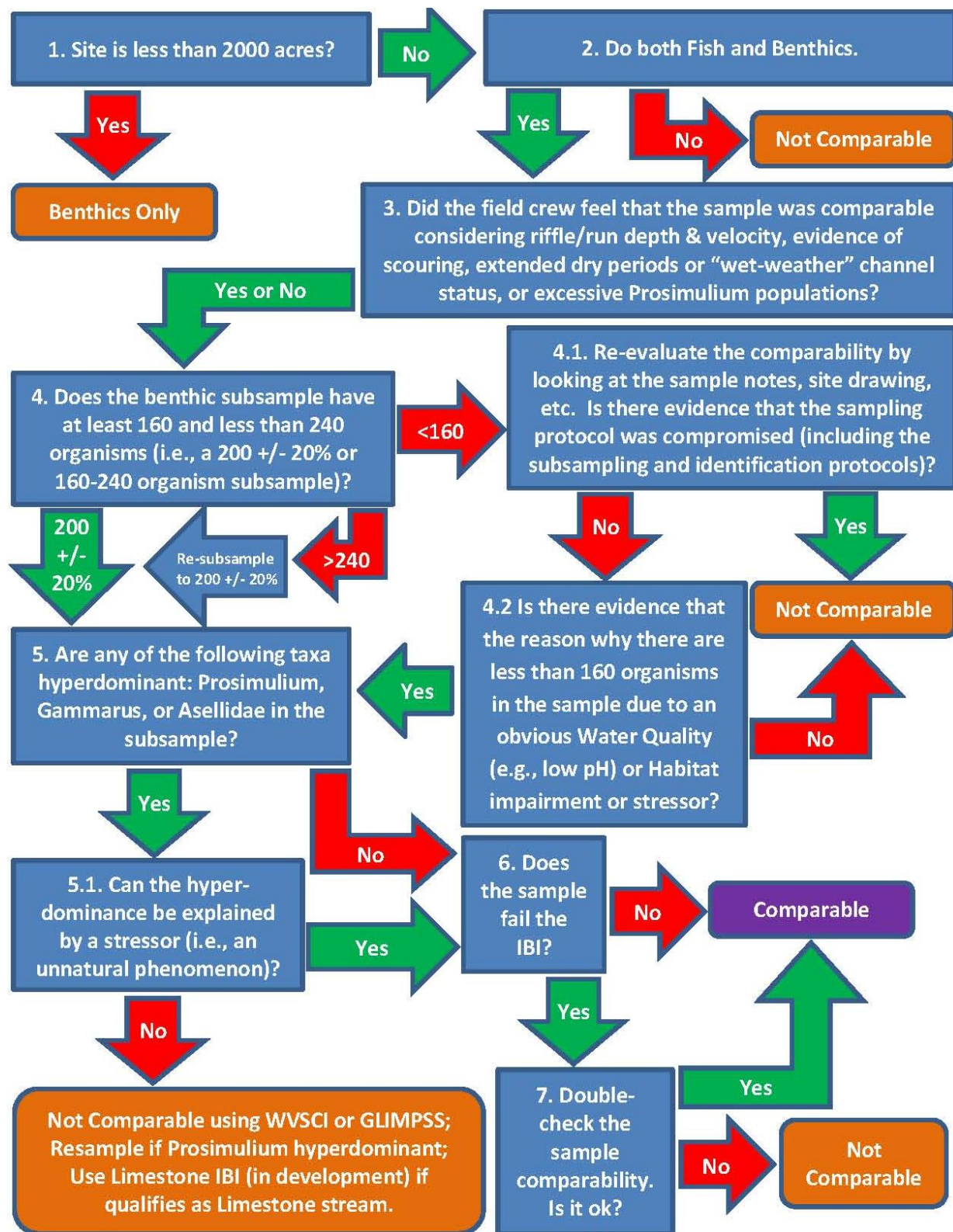
1. Site is less than 2000 acres? —No→ **2. Do both Fish and Benthics.**

1 —Yes→ **Benthics Only**

2 —Yes→ **3.** ; **2** —No→ **Not Comparable**

3. Did the field crew feel that the sample was comparable considering riffle/run depth & velocity, evidence of scouring, extended dry periods or "wet-weather" channel status, or excessive Prosimulium populations?

3 —Yes or No→ **4. Does the benthic subsample have at least 160 and less than 240 organisms (i.e., a 200 +/- 20% or 160-240 organism subsample)?**

4 —<160→ **4.1. Re-evaluate the comparability by looking at the sample notes, site drawing, etc. Is there evidence that the sampling protocol was compromised (including the subsampling and identification protocols)?**

4 —200 +/- 20%→ **5.**

4 —>240→ **Re-subsample to 200 +/- 20%**

4.1 —No→ **4.2 Is there evidence that the reason why there are less than 160 organisms in the sample due to an obvious Water Quality (e.g., low pH) or Habitat impairment or stressor?**

4.1 —Yes→ **Not Comparable**

5. Are any of the following taxa hyperdominant: Prosimulium, Gammarus, or Asellidae in the subsample?

4.2 —Yes→ **5.**

4.2 —No→ **Not Comparable**

5 —Yes→ **5.1. Can the hyper-dominance be explained by a stressor (i.e., an unnatural phenomenon)?**

5.1 —No→ **6. Does the sample fail the IBI?**

5.1 —Yes→ **6. Does the sample fail the IBI?**

6 —No→ **Comparable**

6 —Yes→ **7. Double-check the sample comparability. Is it ok?**

5.1 —No→ **Not Comparable using WVSCI or GLIMPSS; Resample if Prosimulium hyperdominant; Use Limestone IBI (in development) if qualifies as Limestone stream.**

7 —Yes→ **Comparable**

7 —No→ **Not Comparable**

Figure 5-17. Benthic Comparability Flow Chart

Section E. Benthic Macroinvertebrate Data Analysis

Part 1. Genus-Level Index of Most Probable Stream Status (GLIMPSS)

GLIMPSS Reference

A detailed description of the procedures used to develop the Genus-Level Index of Most Probable Stream Status (GLIMPSS) MMI/IBI as well as the steps necessary to calculate final GLIMPSS scores can be found in the following document:

> Pond, G.J., J.E. Bailey, B. Lowman, and M. J. Whitman. 2011. The West Virginia GLIMPSS (genus-level index of most probable stream status): a benthic macroinvertebrate index of biotic integrity for West Virginia's wadeable streams. West Virginia Department of Environmental Protection, Division of Water and Waste Management, Watershed Assessment Branch, Charleston, WV. Available online at:
> *http://www.dep.wv.gov/WWE/watershed/bio_fish/Documents/20110829GLIMPSSFinalWVDEP.pdf*

A reduced version of the GLIMPSS (one that does not address the Winter season or drainages > 60 square miles) was also published in a peer reviewed journal:

> Pond, G.J., J.E. Bailey, B.M. Lowman, and M.J. Whitman. 2013. Calibration and validation of a regionally and seasonally stratified macroinvertebrate index for West Virginia wadeable streams. *Environmental Monitoring and Assessment* **185**: 1515-1540 DOI 10.1007/s10661-012-2648-3. Or on the web at:
> *http://www.dep.wv.gov/WWE/watershed/bio_fish/Documents/20120513GLIMPSSPublicationEnvMonAssess.pdf*

GLIMPSS Overview

WVDEP and USEPA developed this index specifically for use in West Virginia. It was stratified using Season, Region, and Size Class (based on catchment area of the stream). The following are the classifications:

1) **Season** – The seasonal periods are as follows: **Winter** (December-February), **Spring** (March-May), **Summer** (June-October 15). The boundaries between seasons are not hard fast and can vary between years. Because of this, it is recommended to apply a 2-3-week buffer between sampling Seasons to remove seasonal uncertainties. Sampling between October 16 and November 30 (Fall) should not occur due to sampling impracticalities (*e.g.*, leaf fall, hydrology change).
2) **Region** – The state has been divided into two regions: The **Plateau** (corresponding to Ecoregion 70) and the **Mountains** (corresponding to Ecoregions 69, 67, and 66).

NOTE: For samples that are taken on streams that cross regional boundaries, deference may be given to the dominant region of the drainage area above the sample location and the distance to the region boundary over the region in which the sample station is located. For example, a sample taken at the Mouth (Mile 0.1) of Birch River (~38 miles in length) is in Ecoregion 70 (Plateau). However, the watershed above this location is clearly dominated by Ecoregion 69 (Mountains). Additionally, the transition from Ecoregion 69 to 70 is only ~3-5 miles upstream of the mouth. In this situation, a Mountain designation for Region is more appropriate.

3) **Size Class** – Streams are divided into two main size classes: **>60 square miles** and **< 60 square miles**. This is <u>only relevant when</u> sampling in the Mountain region during the Summer season.

Combined, these three stratifications result in 7 different Season/Region/Size Class groupings, here forth referred to as **Seagions** (for Seasonal-Regions):
   A. Winter Plateau
   B. Winter Mountains
   C. Spring Plateau
   D. Spring Mountains
   E. Summer Plateau
   F. Summer Mountains < 60 sq. mi.
   G. Summer Mountains > 60 sq. mi.

NOTE: Due to data restrictions (not a large dataset to work with) the Winter Seasons for both regions were developed using combined Best Standard Values & Worst Standard Values (BSV/WSVs) for both Winter and Spring. However, there was adequate data to set independent reference thresholds for the Winter Season. As more data becomes available, the Winter index will be adjusted using Winter only data to develop Winter specific BSV/WSVs.

Two general versions of the GLIMPSS are available depending on the extent of the Genus-Level taxonomy performed:
   1. **GLIMPSS Chiro Genus or GLIMPSS CG:** All organisms (including all Oligochaeta, Hirudinea, Acari, Mollusca, and Crustacea) should be identified to at least the Genus level except for Hydracarina, Nematoda, and Turbellaria, which can be left at a higher level.
   2. **GLIMPSS Chiro Family or GLIMPSS CF:** This version of the GLIMPSS was developed anticipating the potential limited availability of certified taxonomists who can identify Chironomidae to genera and associated increased costs for identification of samples. The GLIMPSS Chiro Family is identical to the Chiro

Genus version except: 1) the identification of Chironomidae only to family is permitted, 2) Percent Orthocladiinae is replaced by Percent Chironomidae & Annelida in the applicable Seagions, and 3) Percent Tolerant Taxa (TV >6) is not used.

> **NOTE: If the level of taxonomy is restricted to Family for Chironomidae, then only the GLIMPSS Chiro Family can be utilized as the GLIMPSS Chiro Genus cannot be accurately calculated.**

The following GLIMPSS metrics are applied to the benthic data depending the Seagion and version of GLIMPSS used:
- ✓ **Genus Level Taxa Richness** or # Total Taxa
- ✓ **Intolerant Genus Taxa Richness (TV <4)** or # Intolerant Taxa <4
- ✓ **Intolerant Genus Taxa Richness (TV <3)** or # Intolerant Taxa <3
- ✓ **EPT Taxa Richness** or # EPT Taxa
- ✓ **Ephemeroptera Genus Taxa Richness** or # Ephemeroptera Taxa
- ✓ **Plecoptera Genus Taxa Richness** or # Plecoptera Taxa
- ✓ **Trichoptera Genus Taxa Richness** or # Trichoptera Taxa
- ✓ **Clinger Genus Taxa Richness** or # Clinger Taxa
- ✓ **Scraper Genus Taxa Richness** or # Scraper Taxa
- ✓ **Shredder Genus Taxa Richness** or # Shredder Taxa
- ✓ **Modified Genus-Level HBI (Hilsenhoff's Biotic Index)** or HBI
- ✓ **Percent Tolerant Taxa (TV >6)** [#] or % Tolerant Taxa >6[#]
- ✓ **Percent Contribution of Dominant 5 Genera Taxa** or % 5 Dominant Taxa
- ✓ **Percent Ephemeroptera, Plecoptera, Trichoptera (EPT) minus Cheumatopsyche** or % EPT minus Cheumatopsyche
- ✓ **Percent Ephemeroptera** or % Ephemeroptera
- ✓ **Percent Annelida & Chironomidae** or % Annelida & Chironomidae
- ✓ **Percent Chironomidae** or % Chironomidae
- ✓ **Percent Orthocladiinae**[#] or % Orthocladiinae[#]
  [#] Metric only used in Chiro Genus version

> **NOTE: The Richness and Tolerance (including HBI) metrics will vary between the two indices for any one sample since they will have different values depending on the identification level of the Chironomidae considered.**

The Seagion relevant raw metric scores are then standardized on a 100-point scale based on the best standard values and worst standard values (BSV/WSVs) for the given metric. The applicable standardized metric scores are then averaged to give the **GLIMPSS Score** for the sample. Impairment thresholds are set based on the 5th percentile distribution of GLIMPSS scores for a set of reference condition sites in the given Seagion. To compare samples across Seagion, the GLIMPSS score is divided by the Impairment Threshold value for the Seagion resulting in a **Percent of the Threshold** (POT or %OT) for the sample. POT values that scored at or above the threshold value (*i.e.*, Unimpaired) are >=100% and those that scored below the threshold value (*i.e.*, Impaired) are <100%.

Restrictions for Calculating the GLIMPSS

Sample methodology – Identical sampling area (4 x 0.25m^2 kicks = **Total of 1 m^2 area)** and gear (0.5 m rectangular kick-net with a **595-600 μm mesh**) should be used in **rocky riffle/run substrate habitat** *(do not sample pools, undercut banks, or large woody debris)*. Sampling should be restricted to the **Thalweg portions of the channel** (*i.e.*, the deeper portions of the channel that maintain flow the longest) and *should not occur in or near the shallow edges of the channel*. In limited circumstances, 0.3 m d-frame nets with comparable mesh size can be used if total of 1 m^2 total area is sampled.

Comparable samples – The following scenarios should be considered before collecting benthic macroinvertebrate samples for biological health assessments because they are not necessarily associated with human perturbations:
1. Collecting samples following a **drought** may result in reduced organism numbers and diversity. The benthic macroinvertebrates will either have drifted downstream or burrowed deep into the sediments beyond what is normally sampled in kick sampling. In many cases, a stream that has experienced extended dry periods or drought may respond to recent rainfall with a low flow, but the substrate will not have had time to be fully recolonized by benthic macroinvertebrates as it takes a prolonged flow event to break aestivation/diapause.
2. **Low flow** conditions in riffle/runs may affect benthic sampling efficiency by reducing the number of organisms being swept into the net.
3. **High flow** conditions in riffle/runs may affect benthic sampling efficiency by reducing the number of organisms being captured in the net (*i.e.*, they may go over or around the net and the net may quickly become blocked by floating debris and cause back eddies directing material out of the net).
4. **Turbid water** conditions should be avoided for sampling as it could affect sampling efficiency. As a rule, if one cannot see the bottom of the sampling area enough to adequately estimate the substrate composition, one should not attempt a benthic sample and wait for the stream to clear.
5. Collecting samples following a **scour or flood event** may result in reduced organism numbers and diversity.

Seasonality – Acceptable collection dates are from **December 1 to October 15**. This is the time frame of the data that was used to develop the GLIMPSS and any sampling event outside of this window is considered not comparable. It is recommended to apply a 2-3-week buffer between sampling Seasons to remove seasonal uncertainties. Sampling between October 16 and November 30 (Fall) should not occur due to sampling impracticalities (*e.g.*, leaf fall, hydrology change).

Laboratory subsampling – samples in which more than the target subsample size was picked **(200 ±20%)** should be re-sorted to obtain the preferred number of organisms. **As a rule-of thumb, samples containing less than 100 organisms should be closely scrutinized for comparability before calculating a GLIMPSS score.** These

sites may be heavily impacted by stressors (=impairment) or were recently subjected to drought or scour events (=non-impairment).

Taxonomic resolution – Taxonomic resolution for the GLIMPSS is **genus-level except for Nematoda. This includes the non-insect groups like Turbellaria, Mollusca, Oligochaeta, Hirudinea, Acari, and Crustacea.** If higher taxonomy is necessary (*e.g.,* early instar or damaged specimens), then these taxa should not be counted in richness metrics unless they are believed to be distinct from other taxa identified in the sample. WVDEP WAB should be consulted for exact taxonomic resolution of some groups. Collembola is not used with the GLIMPSS.

Tolerance values, Functional Feeding Groups, and Habits – GLIMPSS metrics that rely on **tolerance values** (*e.g.*, HBI), **Functional Feeding Groups** or FFGs (*e.g.*, # Shredder Taxa), or Habits (*e.g.* # Clinger Taxa) are **specifically designated** by WAB and only these designations should be used for a valid GLIMPSS scores calculation.

GLIMPSS Calculations — Use only those **Best Standard Values/Worst Standard Values** (BSV/WSVs) and **component metrics** found in the GLIMPSS development document. Component metrics used for calculating GLIMPSS scores are restricted to those listed above. **Exclusion of any one of these metrics or the inclusion of additional metrics will result in an invalid GLIMPSS score.**

Using the GLIMPSS for Data Analysis

Macroinvertebrate data is evaluated through the preparation of a stream assessment chart. This chart considers the biological and habitat conditions of each stream and compares them to those of the reference sites. Reference sites are those stations having optimal habitat (as defined by the RBP Visual-Based Habitat Assessment scores) and no obvious impairments in water quality. The condition quality of reference sites selected varies depending on such variables as stream size and region. In this case, the framework for these assessments is the GLIMPSS. Stream scores are plotted within a chart and the results are used for watershed assessments (*e.g.*, pollution studies, spill response studies, *etc.*).

Part 2. West Virginia Stream Condition Index (WVSCI)

WVSCI Reference

A detailed description of the procedures used to develop the WVSCI MMI/IBI as well as the steps necessary to calculate final WVSCI scores can be found in the following document:

Gerritson, J., J. Burton, and M.T. Barbour. 2000. *A Stream Condition Index for West Virginia Wadeable Streams.* Tetra Tech, Inc. Owing Mills, MD.

Or on the web at:
http://www.dep.wv.gov/WWE/watershed/bio_fish/Documents/WVSCI.pdf

and an addendum document at:
    http://www.dep.wv.gov/WWE/watershed/bio_fish/Documents/WVSCI Addendum.doc

WVSCI Overview

Tetra Tech, Inc. developed this index specifically for use in West Virginia.

All organisms identified for analysis using the WVSCI (including all Oligochaeta, Hirudinea, Acari, Mollusca, and Crustacea) should be identified to at least the Family level except for Nematoda and Collembola.

The following metrics are applied to the benthic data:
1. **Family Level Taxa Richness** or # Total Taxa
2. **Family Level Ephemeroptera, Plecoptera, Trichoptera (EPT) Taxa Richness** or # EPT Taxa
3. **Percent EPT** or % EPT
4. **Percent Contribution of Dominant 2 Family Level Taxa** or % 2 Dominant Taxa
5. **Percent Chironomidae** or % Chironomidae
6. **Modified Family Level HBI (Hilsenhoff's Biotic Index)** or HBI

The individual metric scores are then standardized on a 100-point scale based on the best standard values (BSVs) for the given metric. The standardized metric scores are then averaged to give the WVSCI (West Virginia Stream Condition Index). A reference threshold is set based on the distribution of WVSCI scores for a set of reference condition sites.

Restrictions for Calculating the WVSCI

A. <u>Sample methodology</u> – Identical sampling area (4 x 0.25m^2 kicks = **Total of 1 m^2 area)** and gear (0.5 m rectangular kick-net with a **595-600 µm mesh**) should be used in **rocky riffle/run substrate habitat** (*do not sample pools, undercut banks, or large woody debris*). Sampling should be restricted to the **Thalweg portions of the channel** (*i.e.*, the deeper portions of the channel that maintain flow the longest) and *should not occur in or near the shallow edges of the channel*. In limited circumstances, 0.3 m d-frame nets with comparable mesh size can be used if total of 1 m^2 total area is sampled.

B. <u>Comparable samples</u> – The following scenarios should be considered before collecting benthic macroinvertebrate samples for biological health assessments because they are not necessarily associated with human perturbations:
1) Collecting samples following a **drought** may result in reduced organism numbers and diversity. The benthic macroinvertebrates will either have drifted downstream or burrowed deep into the sediments beyond what is normally sampled in kick sampling. In many cases, a stream that has experienced extended dry periods or drought may respond to recent rainfall with a low flow, but the substrate will not have had time to be fully recolonized by benthic

macroinvertebrates as it takes a prolonged flow event to break aestivation/diapause.

2) **Low flow** conditions in riffle/runs may affect benthic sampling efficiency by reducing the number of organisms being swept into the net.

3) **High flow** conditions in riffle/runs may affect benthic sampling efficiency by reducing the number of organisms being captured in the net (*i.e.,* they may go over or around the net and the net may quickly become blocked by floating debris and cause back eddies directing material out of the net).

4) **Turbid water** conditions should be avoided for sampling as it could affect sampling efficiency. As a rule, if one cannot see the bottom of the sampling area enough to adequately estimate the substrate composition, one should not attempt a benthic sample and wait for the stream to clear.

5) Collecting samples following a **scour or flood event** may result in reduced organism numbers and diversity.

C. Seasonality – Acceptable collection dates are from **April 15 to October 15**. This is the time frame of the data that was used to develop the WVSCI and any sampling event outside of this window is considered not comparable.

D. Laboratory subsampling – samples in which more than the target subsample size was picked **(200 ±20%)** should be re-sorted to obtain the preferred number of organisms. **As a rule-of thumb, samples containing less than 100 organisms should be closely scrutinized for comparability before calculating a WVSCI score.** These sites may be heavily impacted by stressors (=impairment) or were recently subjected to drought or scour events (=non-impairment).

E. Taxonomic resolution – Taxonomic resolution for the WVSCI is **family level except for Turbellaria, Nematoda, and Collembola. This includes the non-insect groups like Mollusca, Oligochaeta, Hirudinea, Acari, and Crustacea.** If higher taxonomy is necessary (*e.g.,* early instar or damaged specimens), then these taxa should not be counted in richness metrics unless they are believed to be distinct from other taxa identified in the sample. WVDEP WAB should be consulted for exact taxonomic resolution of some groups.

F. Tolerance values – WVSCI metrics that rely on **tolerance values** (HBI) are **specifically calibrated** to those used by WAB and these specific tolerance values should be used for valid final WVSCI scores.

G. WVSCI Calculations — Use only those **best standard values** (BSVs) and **component metrics** found in the WVSCI development document. Component metrics used for calculating WVSCI scores are restricted to those listed above. **Exclusion of any one of these metrics or the inclusion of additional metrics will result in an invalid final WVSCI score.**

Using the WVSCI for Data Analysis

> **NOTE:** **Because of the limitations of the WVSCI MMI/IBI compared to the GLIMPSS MMI/IBI, the WVSCI should only be used when benthic data is available, but further taxonomic identification is not viable (*e.g.*, legacy data from older studies where the voucher specimens are not available for reidentification).**

Macroinvertebrate data is evaluated through the preparation of a stream assessment chart. This chart considers the biological and habitat conditions of each stream and compares them to those of the reference sites. Reference sites are those stations having optimal habitat (as defined by the RBP Visual-Based Habitat Assessment scores) and no obvious impairments in water quality. The condition quality of reference sites selected varies depending on such variables as stream size and region. The framework for these assessments is the West Virginia Stream Characterization Index (WVSCI). Stream scores are plotted within a chart and the results are used for watershed assessments (*e.g.*, pollution studies, spill response studies, *etc.*).

Part 3. Dirty Null Stressor Identification Model

The benthic data is also imported into an analysis model that compares each sample's community structure to that a set of "reference" data with well-known and established stressor types (Metals, Sediment, Ionic Stress, and Reference Condition), also known as "Dirty Nulls". The data that results from the Dirty Null Stressor Identification Model is a set of similarity indexes and probability percentages that help identify potential stressor or stressors to the stream community. The Dirty Null Stressor Identification Model is helpful during TMDL development during the Stressor Identification process.

Benthic Macroinvertebrate Data Analysis Quality Assurance/Quality Control

The samples used in the ***Benthic Laboratory Identification Quality Assurance/Quality Control*** are also analyzed for index performance differences.

Comparison of Index Result (CIR)

Comparison of Index Result is a simple comparison of how the identification differences between taxonomists would affect the MMI/IBI (Multi-Metric Index/Index of Biotic Integrity) score(s) and final impairment decision. To do this you would simply calculate the sample MMI/IBI score from each taxonomist's identification independently and then get the absolute value of the differences. Each MMI/IBI score can then be translated into the appropriate MMI/IBI narrative category (*e.g.,* Unimpaired vs. Impaired, Unimpaired-Very Good vs. Unimpaired-Good, Slightly Impaired vs. Moderately Impaired) to see if the differences in identification between taxonomists result in conflicting decisions about the level of impairment. The absolute value of the differences of MMI/IBI scores can help

qualify the extent of disagreement, especially when the MMI/IBI scores straddle a category threshold.

Additionally, duplicate samples taken by two different samplers at the same time (*see Benthic Sampling Quality Assurance/Quality Control on page 5-14*) are also run thru a Comparison of Index Result analysis to see how two different samplers affect the performance of an index at a site. If the two duplicate samples lead to different narrative categories, then a more in-depth analysis of the field data and notes are performed to see if there was a reason (*e.g.*, sampler error) why the two samples did not match.

Section F. Special Benthic Sampling Considerations-Federally Listed Crayfish

Starting in 2017, special provisions regarding the federally listed Big Sandy crayfish (*Cambarus callainus*) and Guyandotte River crayfish (*Cambarus veteranus*) were included in all Scientific Collection Permits issued by the WV Division of Natural Resources. Specifically, the special provisions state:

1. All benthic macroinvertebrate surveys for certain streams in the Tug Fork and Upper Guyandotte watersheds (USGS 8-digit HUCs) require coordination with the U.S. Fish and Wildlife Service (USFWS). The streams affected by the special provision are provided in *Table 5-1 below and Table 5-2 on page 5-48*.
2. All surveys must have protocols in place to avoid impacts to the listed crayfishes and must be submitted at least via US Mail 30 days prior to the start of sampling and approved by the USFWS before any work can begin.
3. At last one person on the field sampling crew needs to be certified by USFWS and WVDNR for field identification of the federally listed crayfish.
4. All Federally Listed crayfish that are captured are to be photo documented and released.
5. To avoid impacts to crayfish reproduction, no surveys are to be conducted from July 20 to September 10 without permission from USFWS.

Table 5-1. Tug Fork Watershed (05070201 USGS 8-Digit HUC) Streams listed for Endangered Crayfish

Stream Name	AN-Code	NHD AN-Code	Reach Description
Tug Fork	WVBST	BST	Entire Length
Marrowbone Creek	WVBST-19	BST-29	Entire Length
Pigeon Creek	WVBST-24	BST-35	Entire Length
Laurel Fork/Pigeon Creek	WVBST-24-E	BST-35-K	Entire Length
Rockhouse Fork	WVBST-24-Q	BST-35-AF	Entire Length
Buffalo Creek	WVBST-31	BST-45	Entire Length
Mate Creek	WVBST-40	BST-57	Entire Length
Grapevine Creek	WVBST-43	BST-62	Entire Length
Panther Creek	WVBST-60	BST-83	Entire Length
Dry Fork	WVBST-70	BST-98	Entire Length
Bradshaw Creek	WVBST-70-M	BST-98-W	Entire Length
Hite Fork	WVBST-70-M-2	BST-98-W-8	Entire Length
Barrenshe Creek	WVBST-70-T	BST-98-AQ	Entire Length
Jacobs Fork	WVBST-70-W	BST-98-AW	Entire Length
Clear Fork	WVBST-76	BST-106	Entire Length
Spice Creek	WVBST-78	BST-109	Entire Length
Elkhorn Creek	WVBST-99	BST-138	Entire Length
Little Indian Creek	WVBST-100	BST-139	Entire Length
South Fork/Tug Fork	WVBST-115	BST-163	Entire Length

Table 5-2. Upper Guyandotte Watershed (05070101 USGS 8-Digit HUC) Streams listed for Endangered Crayfish

Stream Name	AN-Code	NHD AN-Code	Reach Description
Guyandotte River (Upper)	WVOG-up	OGU	From Taplin Upstream (MP 95.64-Henry Hollow to HW)
Island Creek	WVOG-65	OGU-1	Entire Length
Rum Creek	WVOG-70	OGU-10	Entire Length
Buffalo Creek	WVOG-75	OGU-27	Entire Length
Huff Creek	WVOG-76	OGU-28	Entire Length
Elk Creek	WVOG-80	OGU-34	Entire Length
Gilbert Creek	WVOG-89	OGU-47	Entire Length
Horsepen Creek	WVOG-89-B	OGU-47-B	Entire Length
Little Huff Creek	WVOG-92	OGU-54	Entire Length
Big Cub Creek	WVOG-96	OGU-62	Entire Length
Clear Fork	WVOGC	OGU-70	Entire Length
Laurel Fork	WVOGC-16	OGU-70-X	Entire Length
Indian Creek	WVOG-110	OGU-84	Entire Length
Brier Creek	WVOG-110-A	OGU-84-D	Entire Length
Turkey Creek	WVOG-118	OGU-94	Entire Length
Rockcastle Creek	WVOG-123	OGU-107	Entire Length
Pinnacle Creek	WVOG-124	OGU-108	Entire Length
Still Run	WVOG-130	OGU-124	Entire Length
Barkers Creek	WVOG-131	OGU-128	Entire Length
Slab Fork	WVOG-134	OGU-132	Entire Length
Stonecoal Creek	WVOG-139	OGU-141	Entire Length
Tommy Creek	WVOG-139-A	OGU-141-B	Entire Length

When working in the Tug Fork or Upper Guyandotte watersheds, all streams affected by the federally listed crayfish are tagged and highlighted in any sampling plan involving the collection of benthic macroinvertebrates and communicated to field personnel via the site sampling lists (*see Table 2-1 on page 2-5*). Arrangements are made to ensure that at least one person is present that is certified to identify the federally listed crayfish.

The Watershed Assessment Branch has worked with USFWS and WVDNR to develop a decision matrix to help WAB personnel decide when it is necessary to implement modified benthic sampling protocols to protect the federally listed crayfish (*see Figure 5-18 on the next page*).
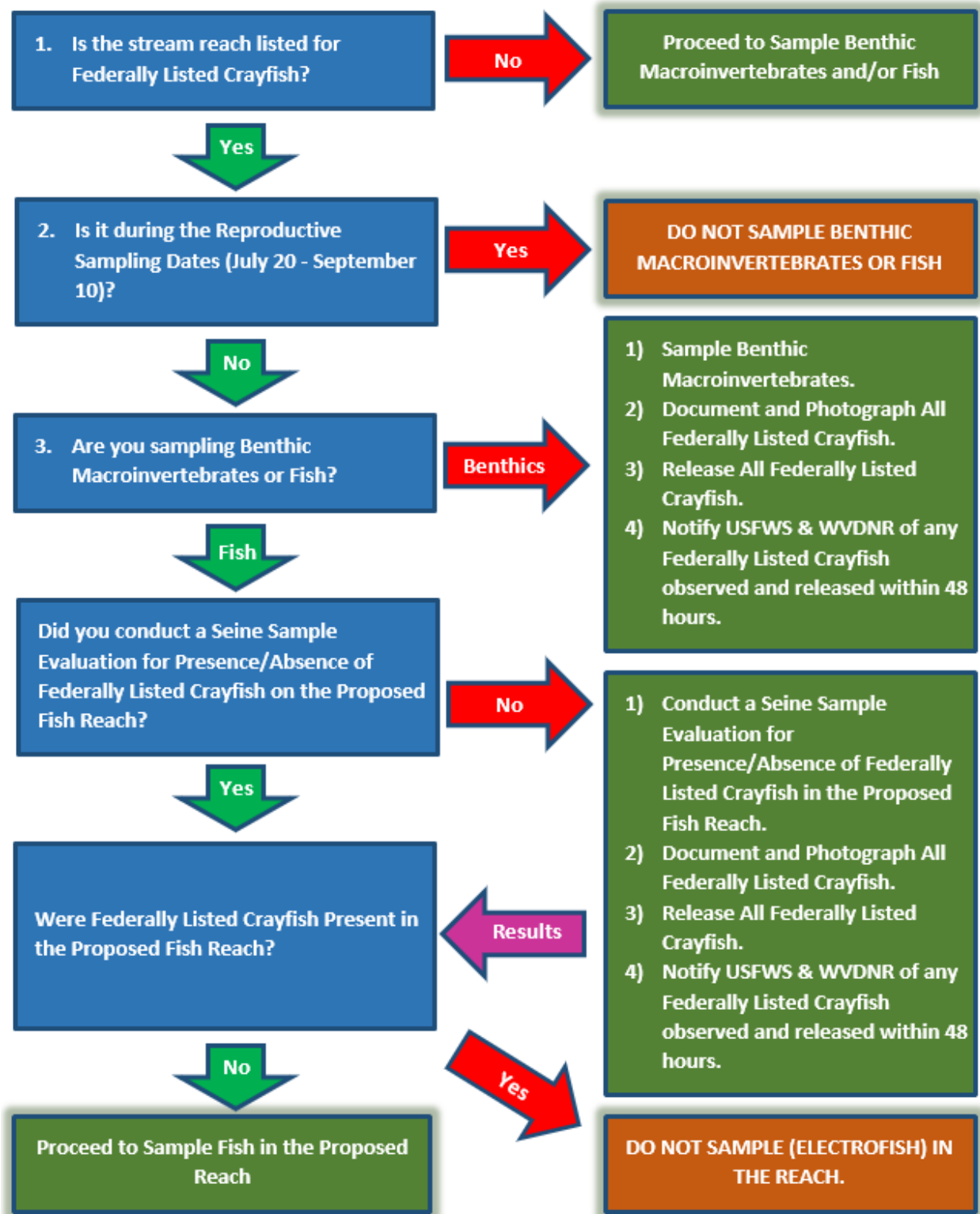
Figure 5-18. Federally Listed Crayfish Sampling Protocol Decision Matrix

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