West Virginia Division of Natural Resources
Scientific Collecting Permit

Standard Conditions for Environmental Assessments on Wadeable Streams

November 10, 2008
Elkins Operations Center

Standard Conditions

1. This permit is valid beginning on the date listed as the "commence date" on the permit application through December 31 of the same calendar year.

2. Collections made under this permit shall be made under the direct and immediate supervision of an individual or individuals certified by the West Virginia Division of Natural Resources (WVDNR) as having completed training in collecting and reporting protocol, unless other arrangements have been made with the WVDNR Scientific Collecting Permit Coordinator.

3. General collecting permits are valid only after the specific collection locations are communicated to the WVDNR. Latitude/Longitude coordinates of the downstream-most collection site accurate to less than or equal to 100 meters and a location description (including county, stream name, named topographic and man-made features and distance from stream mouth) are the acceptable forms of communicating these data. These data will be communicated to the WVDNR Scientific Collecting Permit Coordinator at least 10 days prior to collecting. An addendum will be issued for each project and additional conditions will be incorporated in the addendum.

4. The permit holder shall notify the Scientific Collecting Permit Office
   Ms. Barbara Sargent
   WVDNR, P.O. Box 67, Elkins, WV 26241
   telephone: 304/637-0245
   fax: 304/637-0250
   e-mail: barbarasargent@wvdnr.gov
   and the appropriate district biologist (Table 1) of the time(s) and location(s) where collections will be conducted. The notification shall be made no less than two working days prior to conducting the field work.

5. Collections of aquatic benthos will be made during the time period April 15 - Oct. 15, and fishes will be collected between April 1 and June 15 (inclusive) unless the permittee provides acceptable justification to deviate from this standard.

6. Collections of aquatic benthos will, in general, be made using the Rapid Bioassessment protocol published by the U.S. Environmental Protection Agency (Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. ***Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition***. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.). Sections A, B, and C provide the specific protocols required by this collection permit and have been designed to meet the biological monitoring needs of the: U.S.Army Corps of Engineers – Huntington District; the

WVDEP – Watershed Assessment Branch; the US EPA – Region III Biologists; and the WV DNR.

7. The permittee will provide WVDNR one copy of any reports, papers, articles and presentations generated from data collected under the auspices of this permit. This does not relieve the permittee of any obligations it may have to report to other entities or agencies.

8. Reports on activities should be submitted in appropriate format to the Scientific Collecting Permit Coordinator no later than 45 days following permit expiration.

9. The fish and benthic sub-sample that are taxonomically classified and enumerated will be retained as the voucher sample for each site. Fish and benthic vouchers will be retained until a release is secured from the WVDNR Scientific Collecting Permit Coordinator. WVDNR may request that random voucher collections be provided for examination.

Table 1 – District Biologists

District	Counties	District Biologist	Voice	Fax	E-Mail
1	Hancock, Brooke, Ohio, Marshall, Wetzel, Taylor, Monongalia, Marion, Preston, Harrison, Barbour, Tucker	Frank Jernejcic	304/825-6787	304/825-6270	frankjernejcic@wvdnr.gov
2	Grant, Mineral, Morgan, Berkeley, Jefferson, Hardy, Hampshire, Pendleton	Jim Hedrick	304/822-3551	304/822-7331	jimhedrick@wvdnr.gov
3	Lewis, Upshur, Randolph, Braxton, Webster, Nicholas, Pocahontas, Clay,	Kevin Yokum	304/924-6211	304/924-6781	kevinyokum@wvdnr.gov
4	Raleigh, Fayette, Greenbrier, Mercer, Summers, Monroe, Wyoming, McDowell	Mark Scott	304/256-6947	304/256-6814	markscott@wvdnr.gov
5	Mason, Putnam, Kanawha, Cabell, Lincoln, Boone, Wayne, Mingo, Logan	Zack Brown	304/675-0871	304/675-0872	zackbrown@wvdnr.gov
6	Tyler, Pleasants, Doddridge, Ritchie, Wood, Wirt, Calhoun, Gilmer, Jackson, Roane	Scott Morrison	304/420-4550	304/420-4554	scottmorrison@wvdnr.gov

Section A – Site Information and Physical Habitat

Basic descriptive, supportive and locational data shall be provided for each sample site and/or sampling event.

In order to provide basic descriptions about the physical habitat of the sampling location, it is recommended that at a minimum, the Visual-Based Habitat Assessment procedure be completed. (Section 5.2 from:
Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. ***Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition***. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.)

Photographic documentation of the site shall be provided in digital format.

Section B – Benthic Macroinvertebrates

Basis of Sampling Method

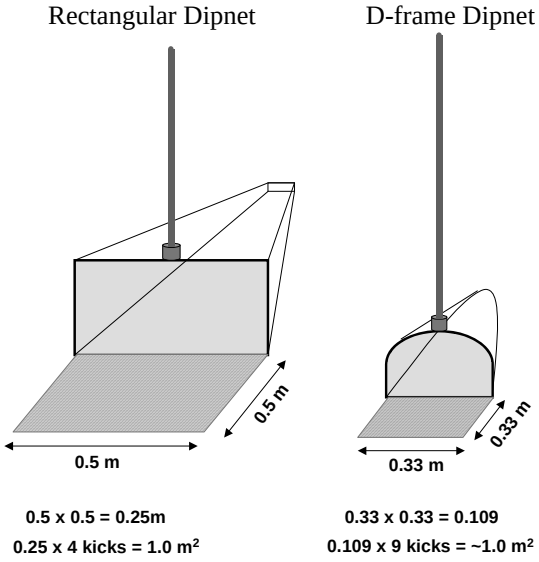
The sampling methods to be used in the WVDEP Watershed Assessment Branch (WAB) are qualitative in nature and are outlined in "Rapid Bioassessment Protocols for Use in Wadeable Rivers and Streams, Second Edition" - U.S. Environmental Protection Agency, July 1999 (EPA 841-B-99-002). This protocol has been adopted for use by many states and organizations. The WAB will utilize the Single Habitat Approach when possible, using a rectangular dipnet (0.5 m wide) or smaller (0.3 m wide) D-net with 595 µm mesh size to sample riffle/run habitats. *It is important to note that the following protocols were established for use by the Watershed Assessment Branch monitoring programs and were intended to provide cost-effective techniques with comparable data across the state. Special projects outside of the Watershed Assessment Branch monitoring agenda (i.e., point source surveys, spills, large river monitoring) may not allow strict adherence to these protocols.*

Selecting Sampling Sites

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 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.

Before sampling begins, a 100-meter assessment reach is established. 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 sampling points with the intent to make collections throughout the entire 100 meters in a diversity of the best available habitats. 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.

MATERIALS AND REAGENTS



1. Rectangular Frame Dipnet – A net with a 0.5 m wide and 0.3 m high frame with 595 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. D-Frame Dipnet - A D-frame (D-net) aquatic dipnet with 595 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 dipnet.
3. Five-gallon bucket - to composite kick samples in the field.
4. 30 mesh sieve (600 µm) - to remove small particulates and water from samples.
5. Small dish washing scrub brush – aid in removing macroinvertebrates from stream substrate particles such as cobble and cleaning the net.
6. Small plastic container or tray – to temporarily hold the organic materials and elutriate.
7. Gallon-sized sample jars - containers to hold benthic sample and associated debris.
8. Inside and outside labels - for sample identification and tracking.
9. Fine-tipped forceps – for removing organisms from net or sieve.
10. One liter squirt bottle – for washing benthic organisms from the bucket, sieve, and elutriate container.
11. 95% Denatured ethanol - for preservation of benthic macroinvertebrates.
12. Ice chest / cooler - for the storage of samples during transport.
13. Sample log book - for tracking the locations of the biological samples.

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.

- Fill the sample jar ½ full with 95% denatured ethanol.
- Check the benthic 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.

I. Rectangular Dipnet (Riffle/Run Habitats = Comparable)

This method is used in streams having riffle/run habitat and a width $\geq$ 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.

1. Select a riffle/run area to sample. Position the net on the stream bottom so as 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.

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

3. Brush the surfaces of all coarse gravel, cobble, boulder, and bedrock substrate. 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. 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.

4. Hold the net handle securely while kicking the substrate vigorously for 20 seconds in an area of about 0.25 m^2 (0.5m wide net x 0.5m upstream) in front of the net. 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.

5. Remove the net from the water with a quick upstream motion to wash the organisms to the bottom of the net. Empty the contents of the net into a five gallon bucket that is partially filled with water. 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.

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. *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 EPA Region III biologists that a 1 square meter sample is adequate for characterizing riffle/run streams in West Virginia where the West Virginia Stream Condition Index 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. 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.

9. Elutriate the bucket's 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). Repeat this process until almost all of 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.

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. 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 material such as fine gravel and sand from the sieve into a sample jar already 1/2 filled with denatured ethanol. Repeat Steps 9-11 until all of 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.

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 organic material (bugs, leaves, CPOM) that was set aside earlier. 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 of 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. Placing the elutriated material on top in the sample jar will protect the organisms from damage due to grinding and compaction during transport o the laboratory. Do not invert or shake the sample jar after the elutriated materials are placed inside.

II. D-net (Riffle/Run Habitat = Comparable)

In some situations the stream may be too narrow or shallow to sample using a Rectangular Dipnet. In this case, a D-net will be substituted for sample collection. The methods outlined for the Rectangular Dipnet 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 ≈ 0.33 m wide, we will sample a square area in front of the net of 0.1108 m^2 (0.333m x 0.333m). In order to sample 1 m^2, we need to collect from 9 locations (0.1108 m^2 x 9 = 0.9972 m^2).

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 of the material in the jar. If more ethanol is needed, it can be added after the sample is received at the laboratory.

2. Place a waterproof label filled out with pencil inside the jar. Include stream name, ANCode, and date. Affix a label to the outside of the jar as well using

clear packing tape. 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.

LAB PROCESSING OF BENTHIC MACROINVERTEBRATE SAMPLES

INTRODUCTION

Benthic macroinvertebrate sample sorting is performed utilizing a modification of U.S EPA's RBP II 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. 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 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. Sample jar - contains the unprocessed sample.
2. Sample vial - for storage of processed sample.
3. Enamel pans - contains sample during the sorting process.
4. Denatured ethanol - preservative used in unprocessed and processed samples.
5. # 30 sieve - used to separate alcohol and fine debris from the sample prior to picking.
6. Gridded sorting tray – a Plexiglas framed sorting tray is used to evenly distribute the washed sample and for randomly selecting the 200 organism subsample. The internal dimension of the tray is 20 inches by 5 inches. There are 100 grids in the tray and each is 1 inch by 1 inch in dimension.
7. Cookie cutter - a homemade cookie cutter, 1 inch by 1 inch is used in conjunction with the sorting tray to isolate each of the subsamples.
8. Labels - Self-adhesive labels are used to identify the contents of the sample bottle (*i.e.*, the picked sample).
9. Tape - used on label as additional adhesive.
10. Pencil - used to label sample bottle.

11. Crucible - or other small container, is used for short term, intermediate storage of the sample during the picking process.
12. Forceps - Fine tipped forceps are used to remove the organisms from the debris.
13. Illuminated magnifier - an optical aid to illuminate and magnify the sample during the picking process. Alternatively, magnifying visors and a desk lamp can be used.
14. Squirt bottle - filled with alcohol, used to rinse organisms into sample bottle.
15. Plexiglas - used to cover sample overnight to prevent evaporation.
16. Counter – used to count the number of organism removed from the sample.

SAMPLE PROCESSING METHODS

1. Select the sample to be sorted. A supervising biologist may provide the picker with a particular sample to be sorted.

2. Select a small bottle that will hold the organisms after sorting is completed. Usually 10 mL bottle is adequate for a 200-organisms sub-sample. A larger bottle may be needed if the sample contains large organisms such as crayfish.

3. Label the bottle:
   a. Use self-adhesive labels
   b. Using a pencil (ink will run if alcohol is spilled on the label), copy all information on the sample jar label onto the self-adhesive label. The label should 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
      - ✓ # of organisms in final sample

   c. Place the label on the bottle and secure with tape.

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 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. Place the tray in a enough water to allow the contents to be swirled and evenly distributed. 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. When the sample is evenly distributed throughout the gridded screen box, remove it from the water.

f. Using a random number generator, select the first grid to be picked. Using the "cookie cutter", isolate the organisms within the chosen grid and scoop the contents of the grid into a white enamel pan. 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

a. Fill a crucible or labeled storage vial with 75% ethanol. If preferred, another small wide-mouth container may be substituted for the crucible.

b. Using fine-tipped forceps and illuminated magnifier or magni-visor, remove all invertebrates from the sub-sample and transfer to the alcohol filled crucible or labeled 10 mL 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.

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 a large number of 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. If 200 or more organisms have been obtained from the initial grid chosen, sub-sampling is complete. 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 159 organisms are obtained or until the entire sample has been picked.

i. Pour the contents of the crucible into the labeled bottle. Use a squirt bottle containing alcohol to rinse the organisms from the crucible. Make sure that all organisms in the bottle 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, if necessary.

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

6. Record Keeping

After a sample has been picked, record the date and your initials in the sample log book.

IDENTIFICATION OF MACROINVERTEBRATES, TAXONOMIC REFERENCES, AND DATA ANALYSIS

Ultimately, the benthic macroinvertebrate data are used to assess the biotic condition of wadeable streams in WV. To accomplish this, a multi-metric index called the West Virginia Stream Condition Index (WVSCI) has been developed. The WVSCI summarizes six biological metrics that represent elements of the structure and function of benthic macroinvertebrate communities. Taxonomic resolution for the WVSCI is family level except for Nematoda and Collembola. **However, all taxa should be**

identified to the genus level or lowest practical taxon. All aquatic macroinvertebrates should be identified including insects, snails, clams, crustaceans (including crayfish), and worms.

MATERIALS AND SUPPLIES

1. Dissecting microscope - for examination of gross features.
2. Compound microscope - for examining minute features.
3. Fine-tipped forceps - for manipulating specimens.
4. Fine-tipped probes - for manipulating specimens.
5. Petri dishes - hold specimens during identification.
6. Alcohol - 75% ethanol is used to preserve the samples and to prevent desiccation during identification.
7. Wash bottle - used for alcohol storage.
8. Microscope slides and cover slips - for examination of tiny specimens and/or body parts under a compound microscope.
9. Benthic macroinvertebrate lab sheet - standard for recording results of identification and enumeration.
10. Taxonomic Keys (see below)

TAXONOMIC REFERENCES

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

Brigham, A. R. 1982a. Coleoptera. In: Brigham, A. R., W. U. Brigham, and A. Gnilka, eds. 1982. Aquatic insects and oligochaetes of North and South Carolina. Midwest Aquatic Enterprises. Mahomet, IL.

Brigham, A. R. 1982b. Megaloptera. In: Brigham, A. R., W. U. Brigham, and A. Gnilka, eds. 1982. Aquatic insects and oligochaetes of North and South Carolina. Midwest Aquatic Enterprises. Mahomet, IL.

Brown, H. P. 1972. Aquatic dryopoid beetles (Coleoptera) of the United States. U. S. Government Printing Office.

Burch, J. B. 1982. Freshwater snails (Mollusca: Gastropoda) of North America. EPA-600/3-82-026.

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

Epler, J.H. 1995. Identification manual for the larval Chironomidae (Diptera) of Florida. Revised edition. Florida Department of Environmental Protection, Division of

Water Facilities, Tallahassee, Florida.

Epler, J.H. 1996. Identification manual for the water beetles of Florida (Coleoptera: Dryopidae, Dytiscidae,Elmidae, Gyrinidae, Haliplidae, Hydraenidae, Hydrophilidae, Noteridae, Psephenidae, Ptilodactylidae, Scirtidae). Florida Department of Environmental Protection, Division of Water Facilities, Tallahassee, Florida.

Epler, J.H. 2001. Identification manual for the larval Chironomidae (Diptera) of North and South Carolina. North Carolina Department of Environmental and Natural Resources, Division of Water Quality, Raleigh, North Carolina.

Huggins, D. G. and W. U. Brigham. 1982. Odonata. In: Brigham, A. R., W. U. Brigham, and A. Gnilka, eds. 1982. Aquatic insects and oligochaetes of North and South Carolina. Midwest Aquatic Enterprises. Mahomet, IL.

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.

Lugo-Ortiz, C.R., and W.P. McCafferty. 1998. A new North American genus of Baetidae (Ephemeroptera) and key to *Baetis* Complex genera. *Entomological News* 109: 345-353.

Merritt, R.W., and K.W. Cummins, eds. 1995. An Introduction to the aquatic insects of North America. 3rd edition. Kendall/Hunt Publishing Company, Dubuque, Iowa.

Merritt, R.W., K.W. Cummins, and M.B. Berg, eds. 2008. An Introduction to the aquatic insects of North America. 4th edition/revised edition. Kendall/Hunt Publishing Company, Dubuque, Iowa.

Peckarsky, B.L., P.R. Fraissinet, M.A. Penton, and D.J. Conklin, Jr. 1990. Freshwater macroinvertebrates of northeastern North America. Cornell University Press, Ithaca, New York.

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

Ross, H. H. 1944. The caddis flies, or Trichoptera, of Illinois. *Bull. Illinois Nat. Hist. Surv.* 23: 1-326.

Smith, D. G. 2001. Pennak's Freshwater Invertebrates of the United States: Porifera to Crustacea. 4th edition. John Wiley & Sons, New York.

Stewart, K. W. and B. P. Stark. 1988. Nymphs of North American Stonefly Genera (Plecoptera). Entomological Society of America.

Unzicker, J. D. and P. H. Carlson. 1982. Ephemeroptera. In: Brigham, A. R., W. U. Brigham, and A. Gnilka, eds. 1982. Aquatic insects and oligochaetes of North and South Carolina. Midwest Aquatic Enterprises. Mahomet, IL.

Unzicker, J. D. and V. H. McCaskill. 1982. Plecoptera. In: Brigham, A. R., W. U. Brigham, and A. Gnilka, eds. 1982. Aquatic insects and oligochaetes of North and South Carolina. Midwest Aquatic Enterprises. Mahomet, IL.

Unzicker, J. D.; V. H. Resh; and J. C. Morse. 1982. Trichoptera. In: Brigham, A. R., W. U. Brigham, and A. Gnilka, eds. 1982. Aquatic insects and oligochaetes of North and South Carolina. Midwest Aquatic Enterprises. Mahomet, IL.

Wiggins, G.B. 1977. Larvae of the North American caddisfly genera (Trichoptera). University of Toronto Press, Toronto, Canada.

Wiggins, G.B. 1996. Larvae of the North American caddisfly genera (Trichoptera). 2nd edition. University of Toronto Press, Toronto, Canada.

White, D. S. 1982. Elmidae. In: Brigham, A. R., W. U. Brigham, and A. Gnilka, eds. 1982. Aquatic insects and oligochaetes of North and South Carolina. Midwest Aquatic Enterprises. Mahomet, IL.

Plafkin, J. L., M. T. Barbour, K. D. Porter, S. K. Gross, and R. M. Hughes. 1989. Rapid bioassessment protocols for use in streams and rivers: Benthic Macroinvertebrates and fish. United States Environmental Protection Agency. EPA/444/4-89-001.

MACROINVERTEBRATE DATA ANALYSIS

1. West Virginia Stream Condition Index (WVSCI)

A detailed description of the procedures used to develop the WVSCI 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. 2000a. *A stream condition index for West Virginia wadeable streams.* Tetra Tech, Inc. Owing Mills, MD. *http://www.wvdep.org/Docs/536_WV-Index.pdf*

2. Restrictions for Calculating the WVSCI

A. Sample methodology – identical sampling area (4 – 0.25m^2) and gear (0.5 m rectangular kicknet with 595µm- mesh) should be used in riffle/run habitat. In limited circumstances, 0.3 m d-frame nets with comparable meshsize can be used as long as 1 m^2 total area is sampled.

B. 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) low flow conditions in riffle/runs may affect benthic sampling efficiency by reducing the number of organisms being swept into the net, (2) collecting samples following drought may result in reduced organism numbers and diversity, (3) high flow conditions in riffle/runs may affect benthic sampling efficiency by reducing the number of organisms being captured in the net, (4) collecting samples following a scour or flood event may result in reduced organism numbers and diversity

C. 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 scrutinized for comparability before calculating a WVSCI score. These sites may be heavily impacted, or were recently subjected to drought or scour events.

D. Taxonomic resolution – taxonomic resolution for the WVSCI is family level except for Nematoda and Collembola. However, all organisms should be identified to the genus level or lowest practical taxon in order to fulfill requirements of the 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.

E. Seasonality– In order for the samples to be comparable to the reference condition established for the WVSCI, samples should be collected between April 15 and October 15.

F. Tolerance values – WVSCI metrics that rely on tolerance values (HBI) are specifically calibrated to those used by WAB and these specific tolerance values (provided within the '*Database for Contractors and Consultants'* Access database provided by WAB) 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 the following:

1. Taxa Richness
2. Ephemeroptera, Plecoptera, Trichoptera (EPT) Taxa
3. Percent EPT
4. Percent Contribution of 2 Dominant taxa
5. Percent Chironomidae
6. HBI (Hilsenhoff Biotic Index)

Exclusion of any one of these metrics or the inclusion of additional metrics will result in an invalid final WVSCI score.

Section C – Fish Collections

Fish collections will be made between April 1 and June 15 (inclusive) unless the permittee provides acceptable justification to deviate from this standard. Large streams (> 3RD order, may be sampled later in the season.) Biological sampling must occur at normal flows.

Fishes will, in general, be collected using EMAP protocols Lazorchak, J. M, D J. Klemm, and D. V. Peck. (eds). 1998. **Environmental Monitoring and Assessment Program-Surface Waters: Field Operations and Methods for Measuring the Ecological Condition of Wadeable Streams**. EPA/620/R-94/004F. U.S. Environmental Protection Agency, National Exposure Research Laboratory, Research Triangle Park, NC 27711. 211pp + 5 Appendices) with the following deviations, clarifications and amendments:

A. The sample reach shall be representative of the stream segment (i.e., will include riffles, runs, pools and glides);,

B. The sample reach will be 40 times the bankfull stream width. The minimum sampling length, in any case, is 150 meters and the maximum length is 500 meters.

C. One (1) backpack electroshocker will be utilized for each 3 meters of sample reach wetted width (up to two electroshockers or six meters). Operators will parallel each other as they advance up the reach. For streams greater than six meters in average wetted width, the pair of operators will zig zag up the reach attempting to collect at each habitat feature. Sampling at all stations will begin and

end on a hydraulic feature (riffle). If this is not feasible, then block nets will be installed at both ends of the sample reach. Some pools, because of size and depth, may not be efficiently sampled by backpack electroshockers. In those cases the field leader should utilize seines to ensure that useful, representative (richness and proportional species representation) samples are collected. On streams > 6 meters in width, a barge or pram electrofishing unit may be used to increase efficiency. A barge may also be necessary in higher conductivity streams (such as those downstream from valley fills) to produce enough power to collect fishes in deeper pools.

D. The following data will be provided from all collections:

1.) the number of each fish species;

2.) an estimate of the maximum and minimum length of each species;

3.) abnormalities / anomalies enumerated by species.

E. All fish collected will be included in the data set.

F. All fishes will be processed and released except for those that are kept for laboratory verification or permanent vouchering:

(1) All rare, threatened and endangered (RTE) species will be examined, a minimum and maximum length estimated and released (note item G., following);

(2) All gamefish will be examined, minimum and maximum length estimated and released (note item G., following);

(3) All conclusively identified fish species will be released following examination, and estimation of minimum and maximum lengths (note item G., following); and

(4) Fish greater than 150mm may be photographed if too large to adequately preserve (photographs should be provided in digital form).

G. Notwithstanding requirements presented in item F- preceding, the following quality assurance and quality control procedures will be followed. In general, guidance provided in USGS Water Resources Investigations Report 98-4239 (Walsh, S. J. and M. R. Meador. 1998. **Guidelines for Quality Assurance and Quality Control of Fish Taxonomic Data Collected as Part of the National Water Quality Assessment Program.** USGS Water Resources Investigations Report 98-4239. Raleigh, NC. 32 pp.) will be followed with these deviations, clarifications and explanations:

(1) With the exception of RTE and game fish species, a voucher collection (> 5 specimens) of each species will be preserved and retained;

(2) RTE species and large specimens (> 150mm) will be vouchered by photographic documentation (photographs which will be used for vouchering purposes shall be provided in digital form);

(3) A sample of small, nongame fish may be retained for laboratory verification of species identification.

(5) Fish and benthic vouchers will be retained until a release is secured from the WVDNR Scientific Collecting Permit Coordinator;

(6) Permitees are encouraged to establish reference collections of fish and benthos in addition to maintaining vouchers and are further encouraged to provide local schools and museums with discarded specimens;

(7) WVDNR may request that random voucher collections be provided for examination; and

(8) Voucher samples will be permanently labeled in a manner that will link the specimens to a specific sampling event.