STANDARD OPERATING PROCEDURES FOR COLLECTION AND ANALYSIS OF BENTHIC MACROINVERTEBRATES

DECEMBER 2013 (Version 4.0)

NORTH CAROLINA
DEPARTMENT OF ENVIRONMENT
and NATURAL RESOURCES
Division of Water Resources
Environmental Sciences Section
Biological Assessment Branch

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This report has been approved for release by:

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1/6/2014

Dianne M. Reid, Chief, Environmental Sciences Section — Date

Date Edited	Editor	Version Edited	Section Edited	Changes/updates
12/16/2013	Michael Walters	Ver. 3.0	Entire document	Division of Water Quality (DWQ) changed to Division of Water Resources (DWR). Biological Assessment Unit (BAU) changed to Biological Assessment Branch (BAB). Updated all headers, footers, and hyperlinks. Updated photos & added labels.
12/16/2013	Michael Walters	Ver. 3.0	Title and Signature pages	Updated document date & version to December 2013 & Version 4.0
12/16/2013	Michael Walters	Ver. 3.0	Signature page	Replaced "Jay Sauber" with "Dianne M. Reid"
12/16/2013	Michael Walters	Ver. 3.0	INTRODUCTION	Removed text: "Estuarine monitoring is no longer conducted by BAB staff " Updated date for prior approved SOP.
12/16/2013	Michael Walters	Ver. 3.0	SAFETY PROGRAM	Removed text: "Sample days longer than 12 hours will not be approved, unless an emergency requires a longer day." Changed Environmental Sciences Branch to Environmental Sciences Section
12/16/2013	Michael Walters	Ver. 3.0	STUDY PLANS	Removed entire section from document
12/16/2013	Michael Walters	Ver. 3.0	SAMPLE COLLECTION	**Sampling Requirements:** Removed text: "Streams less than 1 meter wide should not be sampled. The rule of thumb is that if you can jump across it, you shouldn't sample it." (Note: Small stream criteria now allow assessment of these streams). Removed text: "the trip leader will have an approved study plan or list of sites for basinwide sampling" **Sampling Equipment**: Added section on sampling equipment. **Field Procedures:** Added "inexpensive" to the list of descriptors for benthic sampling equipment.
12/16/2013	Michael Walters	Ver. 3.0	SAMPLING METHODOLOGIES	**Overview**: Added "(i.e. Full Scale)" since that is how we normally refer to the standard qualitative method in reports and other correspondence. Removed text: "that was tested between this revision of the SOP Manual and the last revision". Deleted references to Qual 5 sampling since we no longer use that collection method and have no criteria for assessing samples collected in that manner. **EPT Method**: Replaced the three photos of EPT examples. **Qual 4**: Removed unnecessary background information in this section. Replaced "DA < 3" with "drainage area (DA) < 3.0"
12/16/2013	Eric Fleek & Michael Walters	Ver. 3.0	FRESHWATER SAMPLING TECHNIQUES	Significant revision to descriptions of sampling techniques. **Boat Sampling**: Petite Ponars Replaced "discarded" with "returned to the waterbody" to more accurately describe what to do with large clams picked in the field. **Boat Sampling**: Visuals - *Logs along shore*: Removed text: "unless you want to get very wet".

Date Edited	Editor	Version Edited	Section Edited	Changes/updates
12/16/2013	Michael Walters & Eric Fleek	Ver. 3.0	LABORATORY TECHNIQUES AND DATA INTERPRETATION	Significant revisions to entire section, especially descriptions of methods and criteria. **Introduction:** 1st paragraph - Removed text: "All molluscs and crayfish are saved, labeled, and sent to the museum collections next door." 2nd paragraph - Removed text: "Please use the most current coding memo for the correct codes." 3rd paragraph - Removed text: "outside the summer period" & Changed "Good/Fair" to "Good-Fair" 5th paragraph - Removed text: "and also include habitat evaluation." 6th paragraph - Removed text: "and also include habitat evaluation" **Quality Assurance:** Created a new section for Quality Assurance (no longer under Laboratory Techniques & Data Interpretation). 2nd paragraph – Updated number of taxonomic documents from one to four. Updated the text regarding taxonomic QA to reflect current methods & added Table 17 for guidance. Replaced "log book" with "database" to refer to the storage medium for taxonomic QA results. Inserted a footnote giving web location of taxonomy documents. **Benthic Macroinvertebrate Basinwide Monitoring:** Removed entire subsection from document
12/16/2013	Michael Walters & Joanna Gmyr	Ver. 3.0	Appendices 1-6	Assigned/Updated appendix numbers &updated in-text references. Appendix 1 = regenerated list of tolerance values. Appendix 2 = Field & Lab Equipment list. Appendix 3 = Updated version of the Benthos Collection Card. Appendix 4 = Updated version of the Benthic Lab Sheet. Appendix 5 = Updated habitat form for Mountain/Piedmont Streams Appendix 6 = Updated habitat form for Coastal Plain Streams

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BENTHIC MACROINVERTEBRATES

INTRODUCTION

Benthic macroinvertebrates, especially aquatic insects, are associated with the substrates of streams, rivers and lakes. The Biological Assessment Branch uses aquatic macroinvertebrates as one type of indicator of biological integrity in streams and rivers. A large number of sites are sampled each year during basinwide sampling and special studies. The resulting biological data are used to document both spatial and temporal changes in water quality, and to complement water chemistry analyses. Although bioassessments are useful for identifying biological impairments, they do not identify the causes of impairment. Linking biological effects with their causes is particularly complex when multiple stressors impact a waterbody (USEPA 2000).

There are several reasons for using biological surveys in monitoring water quality. Conventional water quality surveys do not integrate fluctuations in water quality between sampling periods. Therefore, short-term critical events may often be missed. The biota, especially benthic macroinvertebrates, reflect both long and short term conditions. Since many species in a macroinvertebrate community have life cycles of a year or more, the effects of a short-term pollutant will generally not be overcome until the following generation appears.

Macroinvertebrates are useful biological monitors because they are found in all aquatic environments, are less mobile than many other groups of organisms, and are of a size which makes them easily collectable. Moreover, chemical and physical analysis for a complex mixture of pollutants is generally not feasible. The aquatic biota, however, show responses to a wide array of potential pollutants, including those with synergistic or antagonistic effects. Additionally, the use of benthic macroinvertebrates has been shown to be a cost-effective monitoring tool (Lenat 1988). The sedentary nature of the benthos ensures that exposure to a pollutant or stress reliably denotes local conditions, and allows for comparison of sites that are in close proximity (Engel and Voshell 2002).

Analysis of faunal assemblages is one way to detect water quality problems (Rosenberg et al 1986). Different kinds of stress will often produce different benthic macroinvertebrate communities. For example, the taxa associated with organic loading (and low dissolved oxygen) are well known. More recent studies have begun to identify the biological impacts of sedimentation and toxic stress (Burton 1991, Waters 1995, Bode and Simpson 1982, Clements 1994).

Identification at, or near, the species level is desirable for many genera (Cranston 1990, Resh and Unzicker 1975). Such genera may include *Polypedilum*, *Cricotopus*, *Hydropsyche*, *Ephemerella*, *Stenonema, Acentrella* and *Baetis*. Recent work by Lenat and Resh (2001) has shown the benefits of precise taxonomy for both pollution monitoring and conservation biology. Species-level taxonomy is more effective than family-level taxonomy in detecting both the best and worst streams within any given ecoregion. Precise taxonomy is also required to locate the rare species in potential HQW/ORW waters. Tolerant species will usually become dominant only in polluted systems. Allowances must also be made for stream size, geographic location and seasonality. Flow conditions are also related to the relative impacts due to point and nonpoint sources. High flows often increase the impact of nonpoint sources, while reducing the impacts of point sources. The reverse is often true for low flows. Drought conditions can have a more long-term impact on the benthic community than floods. The presence of rare or endangered species is often associated with good water quality.

It is the purpose of this manual to provide details on routine or standard operating procedures of the Biological Assessment Branch (BAB) of the Division of Water Resources (DWR) for the collection and analysis of freshwater benthic macroinvertebrate data. Consistency in data collection and analysis is the cornerstone for evaluating biological integrity. The procedures provided in this manual are a synthesis of widely used methodologies and methodologies developed from the experience of personnel within the unit. These have been shown to provide repeatable and useful data for water quality evaluation.

This manual will be reviewed regularly and revised as necessary. The prior approved version of this manual was dated December 2011. All current employees and new employees within the unit will be provided with this manual to serve as a guideline of the unit's activities, methods, and procedures. Revisions of this manual will be provided to each employee and it will be the responsibility of the employee to keep his or her manual current.

The standard operating procedures (SOP) and quality control procedures (QC) in this manual will be the basis for all benthic monitoring by BAB staff in the waters of North Carolina, and the subsequent data provided in memos and reports. Deviations from these procedures for unusual sampling situations shall be documented in the appropriate report or memo.

SAFETY PROGRAM

The Biological Assessment Branch is required to sample throughout North Carolina at times and places where medical facilities may not be readily available. It is imperative that all employees are instructed in and follow safety precautions when using equipment and hazardous materials. The Environmental Sciences Section has a Safety Committee which is responsible for maintenance and development of current safety procedures. The Committee also maintains the safety standard operating procedures document, with which all personnel should be familiar.

Sampling conditions are the primary safety factor to be considered for field work. If any field conditions, such as high flows or thunderstorms, raise the question of whether a sample can be safely collected, then decisions should always be made with the safety of personnel of prime concern. This same concern for safety of staff must be of primary importance when scheduling the amount of time to be spent in the field. Long days combined with strenuous effort increase the probability of accidents occurring. Safety first must always be the rule.

With the increasing prevalence of Lyme disease and West Nile virus, it is the responsibility of all employees to maximize protection against these insect borne diseases. This should include the use of insect repellants and a thorough check for ticks after every day in the field.

All vehicles are provided with first aid kits, which should be used for minor injuries. Employees should promptly report on-the-job accidents to their supervisor. All employees must be familiar with and follow procedures and deadlines for all Workmen's Compensation claims. If an accident occurs during field operations, the first responsibility of the team leader is to get first aid or emergency treatment for the injured employee; their second responsibility is to promptly notify their supervisor. The Safety Committee maintains a written record of accidents.

SAMPLE COLLECTION

Sampling Requirements

Most of the sampling methodologies described in this manual require that freshwater streams or rivers be wadeable for efficient data collection. High water conditions severely impair sampling efficiency by making some critical habitats inaccessible. An underestimate of taxa richness due to high flows may lead to an incorrect assessment of water quality. **If high water makes sampling conditions marginal, it is better to return to the site during a more appropriate flow regime**.

Drought conditions can also play a major role in altering the composition of the benthic fauna. Every effort should be made in parts of the state that are susceptible to flow interruption during droughts to be sure that flow has been continuous prior to sampling. Flowing water in a stream immediately following a period of rain may mask antecedent conditions. Prior flow conditions can be difficult to determine, especially in smaller streams, but USGS flow data from nearby streams should be used to make the best determination of prior flow conditions. Sampling should be delayed, if possible, when prior flow conditions have been extreme-either high or low.

Before any sampling trip is begun an itinerary will be planned to maximize collection efficiency. Regional Office personnel must be advised before any sampling trip as to where and when work will be done in their region. The trip leader should also use the Internet to check stream stage height from the closest USGS gage station before traveling to the site.

An experienced benthic biologist trained and skilled in field benthic sampling methods and organism identification must be present for all sample collections. New or inexperienced personnel (e.g. staff from other branches of DWR) can be used as team members, if close supervision is provided by the

experienced biologist during sample collection, during sample picking (look through trays again), and during visuals.

Our Endangered Species Permit is renewed annually and requires that **permission be obtained from the Wildlife Resources Commission (WRC) before any sampling be conducted in areas with endangered species**. The back of the permit lists all such areas. If permission is granted, the WRC has also asked that a minimal amount of walking in the stream be done in reaches with endangered mussels, to reduce the possibility of inadvertently crushing the mussels.

Sampling Equipment

Most of the equipment used by the BAB for sampling are in general use by stream biologists across the country and can be readily purchased "off the shelf" from scientific equipment suppliers:

- kick nets with 1000 micron mesh and weighted leading edge
- triangle frame sweep nets with 1000 micron Nitex™ mesh
- sieve buckets with 600 micron mesh (US Standard No. 30)
- forceps
- 6 dram glass vials with polyseal screw caps
- petite ponar (only for boat sampling)

Two pieces of equipment are specialty items:

- sand sampler (special order)
    - rectangular frame net with 300 micron Nitex™ mesh
- fine-mesh sampler (also "chironomid-getter," "midge-getter," or just "getter")
    - 300 micron Nitex™ mesh placed between four inch PVC pipe fittings that are designed to screw together
    - tall round plastic container for EtOH into which the device will fit

Other gear:

- wash tubs (generally available at discount stores)
- picking trays (photographic development trays approximately 36 x 43 cm and 6 cm deep)
- plastic containers with tightly sealing lids large enough to hold several 6-dram vials

Kick Net

Sweep Net

Sand Sampler

Fine-Mesh Sampler disassembled

Fine-Mesh Sampler and Container

Sieve Bucket, Tub, Sample Container

Picking Tray, Vial, Forceps

Benthic macroinvertebrate field equipment is also listed in Appendix 2.

Field Procedures

Samples are collected using the techniques described in this manual. All samples are field picked as described under Standard Qualitative Method. The number of samples collected is dependent on the type of methodology used. Sampling equipment is simple to use, durable, portable, and inexpensive.

Samples are labeled before leaving the site with waterbody name, station location, collection card number, initials of collectors, and date of collection. A gage reading is taken if a gage is present or gage height (stream stage) taken from the USGS web site immediately upon return to the office. Stream stage and stream flow (cfs) should be added to the collection card and entered in the comments section of the

database, along with notes about range of gage heights that should be targeted for adequate sample collection. Photographs of the site must be taken. Water temperature, pH, conductivity and dissolved oxygen measurements will be taken and recorded on the collection card (Appendix 3). All meters must be calibrated in the lab and a lab calibration form filled out, before the meters are taken into the field. Data from an uncalibrated or malfunctioning meter should not be entered into the benthos database. Calibration instructions for all meters can be found in the lab in a notebook with calibration forms.

A site sketch should be made, showing any unique habitats, for all basin assessment locations that do not have site sketches already in the Basin Site Notebooks. This sketch should include enough detail that subsequent samplers can return to the same sampling location every five years.

A habitat assessment form should be filled out for all collections. Directions are given on the form. In most areas, it is obvious whether the Mountain/Piedmont habitat form (Appendix 5) or the Coastal Plain habitat form (Appendix 6) should be used. In some transition areas, however, a field decision must be made as to which form to use. If the stream is naturally rocky with a natural riffle-pool sequence then the Mt/P habitat form should be used, even if the Level IV ecoregion map puts the site in the coastal plain. The reverse is true for a naturally sandy, low gradient stream located on the map in the Piedmont, but near a coastal plain ecoregion.

The benthos collection card (Appendix 3) must be filled out. Field observations should include:

Immediate watershed - type of land use, extent of disturbed land, any floodplain deposition of sediment, any evidence of stream widening and/or filling in, presence of upstream tributaries or dams (including beaver dams), evidence of recent water level changes such as leaf packs out of water, submerged terrestrial vegetation, and sediment on vegetation above water level, any livestock with access to stream, any point sources, any unique habitats.

Substrate - **Two** collectors must make independent estimates of substrate percentages and the independent and average values recorded on the collection card. Also note embedded substrate (interstitial spaces filled in with sand), any atypical habitats such as bridge rubble, large bedrock or other rock outcrops or unusual geological formations, abrupt changes in slope, presence of normal riffle-pool sequence (riffles spaced at intervals equal to 5-7 times stream width), any large areas of unstable coarse sand or movement of bedload material, and amount of substrate covered with *Aufwuchs* or silt.

Width - Since DWR studies have suggested that stream width is a primary factor in determining expected taxa richness, especially in unimpacted headwater streams, the measurement of wetted stream width should be done as accurately as possible. Pacing off a width measurement on the bridge is useful for large rivers. Reflective safety vests should be worn whenever working on bridges. A tape measure could be used to measure smaller streams at two points that are representative of the area sampled. If an actual measurement is not taken, then **two** independent estimates of stream width should be recorded and the average noted, to the nearest whole number. A width estimate of 6.5 meters (average of 6 and 7) implies a degree of accuracy not found with visual estimates. Any unusual characteristics, such as a braided channel in coastal areas, should be noted and recorded.

Water - Look for color, odor (especially sewage and/or chlorine), foaming, algal mats, and oil sheen.

Benthic Community - Note presence of organisms not usually collected such as Bryozoa, sponges, mussel shells. Note dominant organisms and any that are very abundant. Note if diversity is limited to banks and snags above the effects of sediment scour. Give overall impression of site.

All samples are transported in state-owned vehicles to the Biological Assessment Branch in Raleigh. Vehicles are locked when unsupervised and sample custody is maintained at all times by field collectors.

A fixed number of benthic samples are processed at each location. The sampling techniques outlined here usually take 4-6 person hours, i.e. 1.5 - 2 hours per site with three collectors for the standard qualitative method, and 45 minutes to 1 hour for the EPT method using three collectors. However, the time necessary to collect at a station may vary depending on factors such as stream size (a large river takes more time than collecting in a small stream) or flow conditions. A collection team can do a minimum of 3-4 stations per day. Seven stations in close proximity is the record for BAB.

SAMPLING METHODOLOGIES

Overview

Four different macroinvertebrate collection methods are used by the Biological Assessment Branch. The first method is a standard qualitative (i.e. Full Scale) method which can be used to assign water quality ratings to most wadeable flowing streams and rivers in North Carolina. This methodology is applicable for most between-site and/or between-date comparisons, and should be used for all evaluations of impaired streams (those on the state 303d list), that are large enough to rate.

The second collection method is the EPT method, an abbreviated version of the regular qualitative technique. This technique is used to quickly determine between-site differences in water quality. It is particularly useful for watershed or basin assessment studies with large numbers of sites, or emergency sampling where it is desirable to rapidly assess the effect of spills, unusual discharges, etc.

Although the EPT method is a more rapid sampling technique, there are situations where the EPT method may provide too little information for an adequate assessment of water quality. Such situations include areas with naturally low EPT richness and areas where the abundance of more tolerant groups must be assessed. If a biotic index must be calculated, then an EPT sample is inappropriate. In order to decide the most appropriate sampling technique, an investigator must consider the number of sites to be sampled, what kind of existing data might be used for comparisons, how soon a report will be required, and what kind of between-site differences must be detected.

A third sampling methodology is called the Qual 4 method. This uses the same collection techniques as the abbreviated EPT version, but all organisms are picked from the samples. This method should only be used for very small streams that will likely have few EPT taxa, but where data are needed to assess differences in the benthic community.

The fourth collection method is used for swamp streams that stop flowing in summer months, but have visible flow during late winter. A boat sampling technique for sampling nonwadeable freshwater rivers is an adaptation of the standard qualitative method.

Standard Qualitative Method

This collection technique consists of two kick net samples (kicks), three sweep-net samples (sweeps), one leaf-pack sample, two fine-mesh rock and/or log wash samples, one sand sample, and visual collections. Invertebrates are separated from the rest of the sample in the field ("picked") using forceps and white plastic trays, and preserved in glass vials containing 95% ethanol.

Organisms are picked roughly in proportion to their abundance, but no attempt is made to remove all organisms. If an organism can be reliably identified as a single taxon in the field (an example would be *Isonychia*), then no more than 10 individuals need to be collected. A detailed discussion is given below and in Lenat (1988). Some organisms are not picked, even if found in the samples. These include colonial species (Bryozoa and Porifera), Nematoda, Collembola, semiaquatic Coleoptera such as Chrysomelidae, and all Hemiptera except Naucoridae, Belostomatidae, Corixidae and Nepidae. These are not picked either because abundance is difficult to quantify or because they are most often found on the water surface or on the banks and are not truly benthic. The hemipteran families that are included can spend long periods below the water surface.

EPT Method

The EPT technique is a modification of the qualitative collection. The collection and analysis time has been decreased in two ways. First, collections focus on a subset of the benthic community:

Ephemeroptera, Plecoptera, and Trichoptera = EPT

These orders usually include the most intolerant species of benthos. Field notes also are made concerning the abundance of other groups, especially any pollution indicator species. Secondly, the number of collections is decreased from 10 samples (in standard qualitative collections) to only 4 samples: 1 Kick, 1 Sweep, 1 Leaf-pack and "visuals". A comparison of the results between the qualitative and the EPT method is given in Eaton and Lenat (1991).

Qual 4

The Qual 4, as the name implies, is an abbreviation of the standard qualitative method. This method was designed to be used **only** in small streams, which are defined as those sites having a drainage area (DA) $\leq$ 3.0 square miles. In this method four samples are collected: one Kick, one Sweep, one Leaf-pack, and "visuals". All organisms are picked (NCDWQ 2009).

Swamp Method

The Biological Assessment Branch defines “swamp streams” as those streams that are within the coastal plain ecoregion and that normally have no visible flow during a part of the year. This low flow period usually occurs during summer months, but flowing water should be present in swamp streams during the winter months. Sampling during winter, high flow periods provides the best opportunity for detecting differences in communities from what is natural, and only winter (February to early March) benthos data can be used when evaluating swamp streams. The swamp stream must have visible flow in this winter period, with flow comparable to a coastal plain stream that would have acceptable flow for sampling in summer. Swamp streams with pH values of 4.0 or lower cannot be rated, and even those below 4.5 are difficult to evaluate.

The swamp sampling method utilizes a variety of collection techniques to inventory the macroinvertebrate fauna at a site. A total of nine sweep samples (one series of three by each field team member) are collected from each of the following habitat types: macrophytes, root mats/undercut banks, and detritus deposits. If one of these habitat types is not present, a sweep from one of the other habitats is substituted. A sweep for the swamp method is defined as the area that can be reached from a given standing location. Each sweep should be emptied into a tub before the next sweep is collected, to prevent clogging of the net, but all three sweeps can be combined in the same tub. Three log/debris washes are also collected. Visual collections are the final technique used at each site.

Samples are picked on site as described under the Standard Qualitative method above. The primary output for this sampling method is a taxa list with an indication of relative abundance (Rare, Common, or Abundant) for each taxon.

Pre Sample Collection Procedures

One of the most important steps to complete before sampling a site is to survey it from the bridge. Evaluate the upstream and downstream segments for adequate habitat before selecting a reach to sample. Simultaneously examine the area for the safest route to access the chosen sample reach. Consulting the individual site descriptions is an invaluable tool to further aid in this process.

Physical stream form, habitat availability and habitat quality can vary widely between ecoregion. In general, sampling in the piedmont and mountain ecoregions is a straightforward process with good habitat and suitable flow available throughout the sampling reach. Occasionally, in these ecoregions hiking a moderate distance upstream or downstream of the road crossing may be required to find adequate sampling conditions. However, in the coastal plain, it is extremely important to realize that a significant hike upstream or downstream of the bridge pool is often required to find suitable flow and habitat. When working in the coastal plain do not interpret a lack of flow at the bridge pool to be indicative of zero flow conditions within the sampling reach. Hiking upstream or downstream is almost always necessary to find flow in these systems. Consulting the site notes in these (and indeed all areas of the state) is an invaluable tool for saving time in an attempt to find sampleable conditions and easy routes of access to the stream.

Riffle Kick
Equipment: kick net

Approximately two meters of substrate should be disturbed for a typical riffle sample. Rocks that are highly embedded or too large to be dislodged with the foot or leg should be moved by hand if possible. Rocks too large to be moved safely should be manually “scrubbed down” with the hand or foot to dislodge organisms. Careful attention should be made throughout the kick to manually dislodge taxa such as *Neophylax*, *Goera*, *Glossosoma*, *Epeorus*, *Drunella*, (etc.) which are particularly adept at maintaining robust contact with substrates. In addition, if the riverweed *Podostemum*, or other mosses, are present on the substrate in your riffle kick, make certain to “scrub” these “vegetated” surfaces down as numerous unique taxa are associated with these habitats (e.g., *Micrasema*).

When conducting a Full-Scale sample where two riffle kick samples are required, two separate riffle areas should be targeted. If at all possible, avoid taking two riffle samples from the same riffle. Ideally, one riffle kick should be obtained in fast water and another in slower water; avoid taking the two kick samples from the same flow regime. If velocities in all the riffles are the same, obtain a riffle kick from the head of the riffle and one more either mid-riffle or at the end of the riffle. In addition, if *Podostemum* or other mosses are present in riffles, make sure at least one of your samples includes substrate harboring this vegetation. For riffle kicks it may be necessary to walk a considerable distance away from the bridge pool to find a riffle of adequate quality.

In the mountains and most of the piedmont, rocks, cobble, and gravel are typically plentiful and obtaining a “traditional” riffle kick in rocky substrate is straightforward. In the coastal plain (and in some sandy piedmont streams) rocky substrates are either absent or buried. In such instances, riffle kicks can still be obtained by targeting log jams, stick and leaf packs, debris dams, sandy runs and gravel. In such situations, the same general two meters of bottom substrate should be disturbed to obtain the riffle kick.

As in all aspects of benthic macroinvertebrate sampling, extreme caution should be used in obtaining a kick in turbid water or in water that is exceeding base flow during the sample. Aside from the obvious

safety concerns of the collection crew, sampling in turbid water or in discharges exceeding base flow may lead to sampling areas that might have been dry during lower flow conditions. In instances such as these, careful attention should be made to identify the thalweg and concentrate the riffle kick collection in these areas. If you are obtaining a kick near or adjacent to emergent vascular plants you are likely sampling an area that was dry before the water levels increased. If in doubt, do not sample.

Before initiating a riffle kick, make certain the bottom of the net is in full contact with the bottom. If there are gaps between the substrate surface and the bottom of the net, organisms will be missed. This is a particular concern in areas of high velocity. In instances such as this, obtain rocks from outside of the riffle sample area and use them to weight the bottom of the net down. While sampling, it is also a good idea to remove “un-seasoned” fragments of leafs, sticks, as well as trash that are likely not harboring organisms. This will help prevent backflows in the riffle net and will help keep the organisms trapped on the net surface. This can be a particular issue in areas of high flows.

After the sample has been obtained, it is advisable to fold the net over itself to keep taxa from escaping and to keep the net as level as possible until it is placed in the rinse bucket. Remove non-target taxa such as fish and amphibians. Always rinse the net down from the sides and avoid rinsing directly across from the slight opening that is present where the two handles meet. This will prevent taxa from being accidentally rinsed out of the net through this small gap. Rinse until the kick net is free of debris and check for attached organisms. Dump the sample from the bucket into a tub for picking and make sure to wash the remaining debris from the bucket into the tub to ensure all material is retained for examination.

Bank Sweep
Equipment: sweep net

Sweeps of root mats, undercut banks, and macrophytes should target areas of differing flow regimes. Obtaining a sweep from only one zone of current velocity should be avoided and may require a search of the entire reach to find suitable habitat in a variety of current velocities. In waterbodies where there is a lack of well-developed root mats, undercut banks and macrophytes, supplemental sweeps can be made on large bedrock or boulder substrates, particularly if moss or *Podostemum* is present. An effective way to do this is to place the net flush on the substrate below the area of interest and then to mechanically “scrub” the surface with a wading boot or hand. In addition to this supplemental sweep, riffle areas of substrate that were possibly under sampled during the kicks (e.g., small gravel) can also be targeted. Typically, sand or gravel riffles can be kicked by placing the net downstream of the target area and disturbing that portion of the substrate with the foot. In addition, in areas of slack flow (typically near the shoreline) look for silty areas and within these areas of silt deposition look carefully for holes (the diameter of a small nail), which indicate the presence of burrowing mayflies. Running your sweep net a few inches into this silt will likely result in the collection of these taxa.

Given their close proximity to the surface of the water, root mats, undercut banks, and macrophytes are particularly susceptible to temporary drying or brief inundation related to droughts or spates. Therefore, if sampling is being conducted during periods of higher than normal flows, it is imperative to compensate for the temporary inundation of habitat that (while currently wetted) was likely dry before the increased discharge. In such instances, it is advisable to conduct your sweep from depths deeper than normal. If in doubt, do not sample.

Regardless of flow levels, it is always advisable with this sample type to collect from the habitat in a downstream to upstream fashion. This practice will prevent sediment and turbidity from being introduced into downstream unsampled habitat. Sampling in turbid conditions is not recommended, particularly if you can avoid causing turbidity through adaptive techniques such as this. In general, the amount of material collected in the sweep net should be just slightly larger than a softball.

When the sample is complete, dump the material from the net into the tub making sure to backwash the remaining debris from the net into the tub to ensure all material is processed. A careful examination of the net should be made to remove any attached organisms.

Stick & Leaf Pack
Equipment: sieve bucket

Unlike most of the previously discussed sampling types, leaf pack samples are typically restricted to areas of strong flow. As a result, the sampler should not expend too much effort searching for leafpacks in areas of reduced flow unless that is the only flow regime present. Careful attention should be made to the condition of the leaf material comprising the pack. Fresh material should not be targeted. If it is collected together with suitable material, it should be discarded during the elutriation process. Leafpacks should be comprised of well-conditioned material, generally brown to dark brown in color. Black leaves, consistent with anoxic conditions, should be avoided unless that is the only material present. Often when working in the mountains, *Rhododendron* material is the dominant type—particularly in small streams and at higher altitudes. While this material (if properly conditioned) is suitable, it is not ideal and reasonable effort should be expended in obtaining deciduous leaf material. Similarly, when working in the coastal plain and sandhills, American Holly is often a large constituent of leaf pack material and while this is suitable substrate (if conditioned) effort should also be made to target deciduous material whenever possible. Unless it is the only material present, grass clippings and other weedy debris should not be used as part of this sample type.

Obtaining a stick and leaf pack during high flows can be problematic as material currently in flow may actually have been only very recently deposited and thus not consistently exposed to water and not adequately conditioned and likely not to contain any invertebrates. To avoid this, always target leafpacks in the deepest areas of the flow to ensure maximum exposure to water. Avoid taking your entire sample from just one area—it is always best to take multiple samples (regardless of type) from various areas to ensure as representative a sample as possible.

In general, half the volume of the wash bucket is sufficient for a typical leaf pack. More material is acceptable but will result in more time spent in the elutriation process. In either case, elutriation is a crucial step in order to reduce the volume of the sample to a manageable size for picking. To elutriate a sample, submerge the bucket to a maximum of about 2” from the top of the bucket and grab a small hand full of material. Work the material between your fingers while rapidly “washing” it in the standing water of the wash bucket. Washing should be a vigorous process and is intended (along with working the material in your hand) to dislodge invertebrates from the leaf material and to deposit them into the rinse bucket. Repeat this process until the volume of the sample in the bucket is reduced to about 2-3” in depth. During the elutriation process, it is advisable to “rinse” the elutriated material by filling the bucket to within 1” of the top and “twirling” the bucket by the handle rapidly clockwise and counterclockwise until the water is drained. This technique is particularly useful in silty or muddy conditions since rinsing the sample of excessive silt will result in clearer water in the picking tray and thus an easier and more accurate field pick of the sample. In addition the elutriation process also serves to reduce the overall volume of material which gives specimens less refugia for hiding.

Sand Sample
Equipment: sand sampler, fine-mesh sampler, tub

While this sampling method specifically targets sand, in areas where large expanses of sand are not evident (portions of the mountains and slate belt areas in the piedmont), it is advisable to search on the downstream side of large logs and rocks where sand will settle out in the downstream eddy of these objects. Even in streams where sand is obvious, the procedure for collecting this sample remain unchanged: target areas of sand accumulation in a variety of current regimes, place the net down firmly on the substrate downstream of the area you wish to sample, and simple disturb the sand (or the rocks or logs where the sand is accumulated interstitially) and simply allow the disturbed material to collect in the net. When possible, avoid collecting areas of heavy organic deposition (leaf packs, sticks, *Podostemum*) as heavy collections of these materials will make the elutriation and filtering of the sample more problematic. Approximately a soft ball sized amount of material will suffice for this sample type. To elutriate, simply dump the contents of the net into a wash tub (making certain to back wash the net to clear all debris for processing), add some water and swirl the material around several times and then quickly pour off the suspended sediment (and organisms contained within) through the chironomid getter.

Repeat this step at least twice. After the second elutriation, it is acceptable to dump a small portion of the remaining sample before starting the elutriation cycle over again. Repeat this process until the sample has been completely filtered. The remaining sample collected in the bottom of the getter should be approximately the thickness of your thumb.

Rock & Log Wash
Equipment: fine-mesh sampler, tub

The primary purpose of this sampling method is to target substrate sizes and flow regimes that were either missed or undersampled in the previous sample types. For example, although a riffle kick may have been obtained from cobble; large boulders might not have been sampled during the kick. Similarly, perhaps the riffle kicks were taken in substrate that lacked *Podostemum*, or (due to the difficulty in working the kick net in extremely fast flows) the kick was only taken in areas of moderate flow. The rock and log wash is an opportunity to target the largest rocks or logs that can be worked, and in areas of flow (either very fast, or very slow) that are not generally focused on during the other sampling types. Although larger substrates are generally the favored type of habitat for this sample, smaller substrate sizes are also required for a representative sample.

The substrate is placed into a tub and mechanically washed with a combination of splashing water onto the material and rubbing the substrate with the hand and then rinsing the substrate again. Careful attention should be made to ensure the resulting wash water is being collected in the tub. If the substrate is too large to physically place in the tub, washing portions of it at a time over the tub is acceptable. This is particularly easy to execute with large logs where sections can be moved over the tub sequentially until the entire length of the log has been addressed.

After each piece of substrate has been washed, the resulting mix of material and water are poured through the “getter”. In areas of abundant silt, sand or *Aufwuchs*, it may be necessary to facilitate the filtering by unclogging the filter screen. The best way to do this is to strike the top of the getter with the tub. Manually scraping the receiving surface of the filter with a finger can also be an effective way to prevent clogging.

This sampling process is repeated until approximately one thumb width of material has been retained on the filter screen. The actual quantity of substrate required to obtain this volume of material is often related to the productivity of the site. Highly eutrophic conditions may yield this amount of material from just 3-5 pieces of substrate. In situations such as these, care should be exercised and only the best pieces of substrate should be selected for washing. Conversely, in highly oligotrophic waterbodies it may take 15 or more pieces of substrate to approach the recommended volume of material and in such cases the sensitivity of substrate selection is less crucial since so much of it is by necessity being washed. At the conclusion of the sample, simply place the getter into a container with enough ETOH to inundate the collected material. This should be allowed to “cure” for at least 15 minutes before picking. This process will ensure the small chironomids will be motionless and will cause most to float in the surface tension which will make the organism easier to collect.

Visuals
Equipment: forceps, vial filled with 95% EtOH

Each of the three person collecting crew participates in this portion of the bioassessment. The philosophy of this sample type is to specifically target habitats that were either not sampled from any of the aforementioned sample types or were undersampled. Examples generally include very large substrates (e.g., logs and boulders) in areas of very high or very low flow. Essentially, microhabitats are the intended target of this sample type. For example, although multiple substrates in pools or slow runs may have been washed, some highly cryptic taxa (e.g., *Polycentropus*, *Nyctiophylax*, *and Ceraclea*) are still often missed. During visuals, the undersides of rocks in pools and areas of slow flow should be targeted for *Polycentropus* and *Ceraclea* while the cracks and crevices in rocks in the same areas should be examined closely for *Nyctiophylax*. Rotate the rock in multiple orientations to make maximum use of ambient light and to reduce glare. This will promote finding small or cryptic taxa. Also, for small organisms it is often helpful to gently splash water onto substrate surfaces as the disruption of the brief surface tension will reveal the presence of many small organisms that are otherwise hard to see.

In areas of high to medium flows pay close attention for the presence of mineral-cased caddisflies, again often recessed into rock crevices (e.g., *Neophylax*) or sometimes found firmly adhered to the surface of the rock (e.g., *Glossosoma*). These taxa are often not dislodged during the kick or wash sample and so effort must be made to target them during visuals. Similarly, many mayflies (e.g., *Epeorus*) which are adapted to clinging tightly to surfaces in fast current will sometimes not be dislodged during the riffle kick or wash and thus must be searched for during visual collections. Although not necessarily restricted to just areas of fast flow, careful attention should be focused on the presence of *Leucotrichia pictipes* on rock surfaces (particularly in areas of prolonged sun exposure) as well as the presence of the very small caddisfly *Hydroptila*.

Approximately 10-15 minutes should be allotted for this collection. Often, in areas of very poor habitat or very poor diversity, 10 minutes will provide more than enough time to obtain a representative sample. In areas of high habitat heterogeneity or very high diversity, 15 minutes may not allow for enough time to obtain a good sample. It is strongly advisable to pack at least three extra vials to set aside solely for the collection of visual samples and to avoid using the vials employed for the collection of the rest of the sample; this prevents sample loss in the event of a dropped vial. Visuals should be collected at the end of the assessment in order to facilitate the location of undersampled habitats.

Sample and Post Sample Collection Procedures

Excessive sample processing times in the laboratory are an important consideration. Thus, to reduce processing times it is critical to minimize the amount of organic material introduced in the sample. Picking through detritus in the lab in an effort to extract and identify specimens from this material increases processing times. Therefore, while picking in the field, it is extremely advantageous to reduce the amount of this material included in the sample. Moreover, to further reduce both field collection and laboratory processing effort, it is important during the field pick to communicate effectively to avoid over-picking abundant and obvious taxa.

Perhaps one of the most important steps to complete before leaving a site is to make absolutely certain that there are no organisms remaining adhered to collection or processing surfaces. Under no circumstances should organisms from one site be transferred to another site. To prevent this, a systematic examination of all the nets, buckets, trays, tubs, and chironomid getters must be completed before leaving the site. In addition, an inspection of the nets, bucket sieves and chironomid getters should be made at the conclusion of each site to ensure that no tears in the net material are present.

Before packing the sample away for the rest of the sample trip, a quick inspection of the individual vials should be made to assure that no more than about 50% of the vial is comprised of specimens. If the volume of specimens exceeds this general guideline, the sample will not be properly preserved and this can lead to deterioration though decomposition of the specimens in the sample resulting in a reduction in the accuracy of the identification of the specimens contained in the sample. If the vial is overly full with specimens, the volume of the sample should be reduced by placing a portion of it into the larger collection container.

Boat Sampling

Most collections are in wadeable streams, but there are some locations where a boat is required. These are usually large coastal plain rivers, including the lower sections of the Alligator, Chowan, Meherrin, Neuse, Pasquotank, Perquimans, Roanoke, Tar, South, Black, Waccamaw, Wiccacon, Northeast Cape Fear and Cape Fear rivers. In such habitats, petite ponar dredge sampling replaces kick-net samples, but all other standard qualitative collection techniques are still useable. Most of these localities have little or no visible current, but it is important to record in the field notes how much current is present, especially after heavy rainfall. Coastal B criteria are used to evaluate such sampling sites.

The standard boat method still aims at a total of 10 composite samples per site. Efficiency is maximized by leaving 1-2 people on shore to collect sweeps, epifaunal collections, visuals, part of leaf-pack/debris sample, while the boat samplers collect petite ponar samples, at least part of leaf-pack/debris sample, part of one epifaunal wash, and part of visuals (logs in the current). When the shore area is very steep, some sweeps may be collected from the boat, although this can be less effective than wading.

Petite ponars will be collected at 3 locations between midstream and the bank, with three replicates at each location (a total of 9 samples). Sandy samples should be elutriated and processed through a fine-mesh sampler (chironomid getter). Samples that are mainly organic can be picked live, but some portion should be processed through the fine-mesh sampler. If possible, the 3 locations should include a variety of depths, with at least one location in the 2-3 meter range. This may not be possible in all locations; but it is preferable to utilize a variety of depths. No petite ponars should be collected from the area normally sampled during shore work, i.e., <2 meters in depth. The petite ponar should be lowered slowly, so as to avoid disturbance of surface sediments. The shallow collections are often good habitat for *Hexagenia* and *Phylocentropus*. Collection card notes should include some record of the depths sampled and the general substrate composition at each location. Large clams (*Corbicula*, *Rangia*) can be identified, recorded on the collection card, and returned to the waterbody.

Sweeps Three sweeps will be collected from bank habitats at each site, sampling as much of the edge habitat as possible. If aquatic macrophytes are present, then these should be sampled in one of the three sweeps. Other areas to be included include roots and areas of debris. Many kinds of invertebrates are collected this way, but look for cased Trichoptera (*Triaenodes*, *Oecetis*, etc.) and Baetidae.

Leaf packs/Debris (1 composite sample) Leaves and other large particulate organic matter are to be rinsed in a wash bucket. It will often be necessary to use the boat to get to habitats where leaves accumulate. Where leaf packs are not present, then sticks, logs, and aquatic plants may be sampled.

Epifaunal collections (2 composite samples) Macrophytes and well-colonized logs (both in the current and along the shore) should be washed down and processed through the fine-mesh sampler. As usual, this is aimed at getting a good sample of the midge community, but a wide variety of other taxa also will be collected. Collections which have very few numbers of midges should be repeated, as the epifaunal community can be very patchy. If the epifaunal community is very sparse, it is important that it is known that this pattern is related to water quality/habitat quality, and is not a function of sampling technique.

Visuals (treated as 1 composite sample) A fairly large proportion of the EPT fauna often is collected during the visual portion of sampling. Areas to be covered during visuals include:

Macrophytes, especially those with floating leaves. Look for those with some evidence of breakage and/or decomposition. Often the plants on the outside of a macrophyte patch (away from the shore) will have more types of macroinvertebrates. Look for leaf-mining midges and beetle larvae, Hydroptilidae (several genera), snails, and limpets.

Logs along the shore. Look for evidence of long-term colonization, especially periphyton and sponge growths. If the water level has risen recently, it is necessary to search for logs in deeper waters. This often means kicking up logs with your feet. Look for leeches (especially under bark, Polycentropodidae (several genera), small sand-cased Trichoptera (e.g. *Ceraclea*, *Oecetis*, *and Phylocentropus*), *Pycnopsyche*, Heptageniidae, wood-mining midges, and snails. It is crucial that team members can recognize polycentropodid retreats.

Logs in the current. This part of the visuals usually must be conducted from the boat, and should be continued until several well-colonized logs have been found. You should be looking for epifaunal habitat that is out in the current (or where current might be at higher flows), but is large enough not to be washed downstream. This often means dragging into the boat some very large logs; if you can lift it up easily, it is probably too small. Colonization by Hydropsychidae is a good sign, but also look for Heptageniidae, Baetidae, Plecoptera (esp. *Acroneuria* and *Neoperla*), and sand-cased Trichoptera.

LABORATORY TECHNIQUES AND DATA INTERPRETATION

When a sample is returned to the laboratory for analysis, the person identifying the sample will combine all vials collected from a site into one Petri dish for identification. All organisms in the sample are then identified to the lowest possible taxonomic level, recorded on a Benthic Macroinvertebrate Lab Sheet (Appendix 4), and tabulated as Rare=1 (1-2 specimens), Common=3 (3-9 specimens) or Abundant=10 ($\geq$10 specimens). Most organisms may be identified using only a dissecting microscope, but Oligochaeta, Chironomidae and some mayfly structures must be mounted on glass slides and identified with a compound microscope. Following identification, samples are labeled and stored for an indefinite time period. Lab sheets and all associated information are also filed by river basins.

After the sample is identified and the lab sheet is complete, all taxonomic data, along with data from the benthos collection card, is entered by biologists into a benthos database utilizing the software application Microsoft Access. After the data is entered, it is checked for coding or relative abundance errors. It is imperative that consistent coding be used when entering data in the fields for waterbody, sample type, ecoregion and bioclassification. When the data is saved, total taxa richness, EPT taxa richness, Biotic Index value for the sample, EPT Biotic Index value and EPT abundance are automatically calculated. A species list for one or many samples can be retrieved using this system.

The ultimate result of a benthos sample is a bioclassification for the sample. Bioclassifications used by BAB are Excellent, Good, Good-Fair, Fair or Poor for standard qualitative and EPT samples. This bioclassification is automatically calculated in Microsoft Access, unless the sample is from a small stream, or from a swamp stream. Any seasonal corrections are made manually (outside the database) after all taxa in a sample are entered into the database. The bioclassification is entered manually based on the corrected values and notes about corrections are made in the comments section for each sample.

For streams in the mountain and piedmont ecoregions with drainage areas $\leq$ 3 square miles, the Qual 4 method is employed and biocritiera have been developed for these streams (NCDWQ 2009).

The final swamp stream criteria use a three bioclassification approach for evaluation rather than the five classes used for flowing streams because of the higher natural variability found in swamp streams. This variability makes it more difficult to evaluate minor changes in the benthic community. The final bioclassifications or stress categories for swamp streams are Natural, Moderate, and Severe.

A complete list of all benthic macroinvertebrates collected (BINDEX) is maintained in the Microsoft Access database. The BINDEX list contains the taxa code, the species name, order, family, tolerance value (an index based on the pollution tolerance of each taxa), and feeding type of each taxa. This list is given in Appendix 1 for all taxa that have been assigned a tolerance value. Tolerance values (Appendix 1) were updated in April 2010 and followed procedures established in Lenat (1993).

North Carolina Biotic Index

The North Carolina Biotic Index is modeled after the Hilsenhoff Biotic Index (Hilsenhoff, 1987) using tolerance values derived from BAB collection data. The Biotic Index for a sample is a weighted average of the tolerance values for the organisms identified from the sample with respect to their abundance. The biotic index value, scaled from 0 to 10, represents the relative tolerance of the benthic community to the presence of general stressors, with lower values indicating more pristine conditions and higher values indicating stress. Biotic indices may be calculated for the entire benthic community (where it is identified as NCBI or BI) or just the portion of the community represented by the insect orders Ephemeroptera, Plecoptera, and Trichoptera (identified as EPTBI).

The BI value is determined by:

$$B = \frac{\sum(T_i)(n_i)}{N}$$

where

B = the biotic index (BI)
T_i = the tolerance value (TV) for the ith taxon
n_i = the abundance value (1, 3, or 10) for the ith taxon
N = sum of all abundance values

Criteria for Determining Bioclassifications

North Carolina has a suite of criteria used to determine a classification for a stream site. The criteria set used depends upon several factors, including: stream size, flow regime, season of collection, and collection method. Use the following key for direction to the appropriate assessment method:

1. Site is located in the coastal plain *AND* does not normally flow during a portion of the year..... 2
   Site is located either in the mountains or piedmont *OR* normally flows during the entire year 3

2. Swamp collection method was used *AND* sample was collected during February or the first fifteen days of March .....**evaluate using Swamp criteria, page 21**
   Not as above .....**report bioclassification as "Not Rated"**

3. Site is on an unwadeable river on the coastal plain and was collected using Boat method .....**use Coastal B criteria for provisional classification but report as "Not Rated", page 20**
   Not as above 4

4. Site is within the Triassic Basin level 4 ecoregion .....**report bioclassification as "Not Rated"**
   Site is outside of the Triassic Basin 5

5. Drainage area is less than or equal to 3.0 square miles *AND* site is located in either the MT or P Level 3 ecoregion or Sand Hills Level 4 ecoregion *AND* sample was collected during the months of April through June *AND* Qual-4 collection method was used..... ..... **evaluate using Small Stream criteria, page 19**
   Not as above 6

6. Site is located in the mountains *AND* drainage area is less than 3.5 square miles *AND* site is within an undisturbed drainage *AND* either EPT or Qual-4 collection method was used .....**evaluate using High Quality Small Mountain Stream criteria, page 20**
   Not as above 7

7. Drainage area is greater than 3.0 square miles..... 8
   Drainage area is less than or equal to 3.0 square miles..... 9

8. Full Scale collection method was used..... **evaluate using Full-Scale criteria, page 18**
   Either EPT or Qual-4 collection method was used ..... **evaluate using EPT criteria, page 17**

9. Either EPT or Qual-4 collection method was used .....**see Unrated Small Streams, page 20**
   Not as above .....**report bioclassification as "Not Rated"**

EPT Criteria

EPT criteria are appropriate to use when the following conditions are met:

- EPT or Qual-4 collection method was used
- sample was collected from a coastal stream that normally flows throughout the year, or from a mountain or piedmont stream
- drainage area above the site is greater than 3.0 square miles
- sample was not collected from the Triassic Basin level IV ecoregion

If the sample was collected between June and September inclusive, use Table 1 to look up the bioclassification based upon the number of EPT taxa present in the sample and the ecoregion from which the sample was collected. Note that sites in the Sand Hills are within the coastal plain and should be evaluated as such.

Table 1. EPT thresholds for determining bioclassifications using EPT criteria.

	Mountain	Piedmont	Coastal Plain (CA)
Excellent	>35	>27	>23
Good	28-35	21-27	18-23
Good-Fair	19-27	14-20	12-17
Fair	11-18	7-13	6-11
Poor	0-10	0-6	0-5

A seasonal correction may be required for samples collected outside of the summer sampling season (i.e. October through May). Ideally, a nearby reference site with prior summer data and similar site characteristics (e.g. drainage size, substrate, level IV ecoregion) is sampled about the same time as the site to be evaluated. Using the data from this reference site, the adjustment between summer and the seasonal sample (the difference in the number of EPT taxa between the two sampling events at the reference site) can be determined. In the absence of data from a nearby reference site, the number of winter/spring Plecoptera collected are subtracted from the total EPT collected (Table 2).

Table 2. List of Plecoptera taxa used in seasonal adjustments.

Family	Latin Name
Nemouridae	*Amphinemura* spp
	Prostoia spp
	Shipsa rotunda
	Soyedina spp
Taeniopterygidae	*Strophopteryx* spp
	Taeniopteryx burksi
	Taeniopteryx lita
	Taeniopteryx metequi
	Taeniopteryx parvulus
	Taeniopteryx spp
	Taeniopteryx ugola
Chloroperlidae	*Alloperla* spp
	Haploperla brevis
	Haploperla fleeki
	Haploperla spp
Perlidae	*Perlinella drymo*
Perlodidae	*Clioperla clio*
	Cultus decisus complex
	Cultus spp
	Diploperla duplicata
	Diploperla morgani

Family	Latin Name
Perlodidae (cont'd)	*Diploperla* spp
	Helopicus bogaloosa
	Helopicus spp
	Helopicus subvarians
	Isoperla bilineata gr
	Isoperla burksi
	Isoperla dicala
	Isoperla frisoni
	Isoperla lata
	Isoperla nr *holochlora*
	Isoperla nr *namata*
	Isoperla nr *richardsoni*
	Isoperla nr *slossonae*
	Isoperla orata
	Isoperla similis gr
	Isoperla sp A
	Isoperla species 10
	Isoperla spp
	Isoperla transmarina gr
	Remenus bilobatus

To determine the bioclassification for a sample collected outside of the summer seasonal window, subtract the number of seasonal EPT taxa (as determined either by using a nearby reference site or consulting the list of winter/spring Plecoptera) from the total EPT, then look up the bioclassification for the corrected EPT in Table 1.

NOTE: Whether the collection method was EPT or Qual-4, enter as EPT to the database. The BAB benthos database will correctly report the bioclassification for summer EPT samples, as well as for non-summer samples if the number of seasonal EPT is entered.

Full Scale (Standard Qualitative) criteria

Full Scale criteria are appropriate to use when the following conditions are met:

- Full Scale collection method was used
- sample was collected from a coastal stream that normally flows throughout the year, or from a mountain or piedmont stream
- drainage area above the site is greater than 3.0 square miles
- sample was not collected from the Triassic Basin level IV ecoregion

If the sample was collected between June and September inclusive, use Table 3 to look up scores for the biotic index value and the number of EPT taxa collected for the sample. Note that sites in the Sand Hills are within the coastal plain and should be evaluated as such.

Table 3. Thresholds for determining BI and EPT scores using Full Scale criteria.

Score	BI Values			EPT Values		
	MT	P	CA	MT	P	CA
5	<4.00	<5.14	<5.42	>43	>33	>28
4.6	4.00-4.04	5.14-5.18	5.42-5.46	42-43	32-33	28
4.4	4.05-4.09	5.19-5.23	5.47-5.51	40-41	30-31	27
4	4.10-4.83	5.24-5.73	5.52-6.00	34-39	26-29	22-26
3.6	4.84-4.88	5.74-5.78	6.01-6.05	32-33	24-25	21
3.4	4.89-4.93	5.79-5.83	6.06-6.10	30-31	22-23	20
3	4.94-5.69	5.84-6.43	6.11-6.67	24-29	18-21	15-19
2.6	5.70-5.74	6.44-6.48	6.68-6.72	22-23	16-17	14
2.4	5.75-5.79	6.49-6.53	6.73-6.77	20-21	14-15	13
2	5.80-6.95	6.54-7.43	6.78-7.68	14-19	10-13	8-12
1.6	6.96-7.00	7.44-7.48	7.69-7.73	12-13	8-9	7
1.4	7.01-7.05	7.49-7.53	7.74-7.79	10-11	6-7	6
1	>7.05	>7.53	>7.79	0-9	0-5	0-5

Seasonal corrections are required for samples collected outside of the summer (June through September) sampling season. To derive the seasonally corrected EPT richness value, refer to the discussion of seasonal corrections in the EPT Criteria section above. To get a seasonally corrected BI value, add the value from Table 4 for the appropriate season and level III ecoregion to the BI value as determined from the sample.

Table 4. BI corrections for non-summer data.

	Mountain	**Piedmont**	**Coastal Plain (CA)**
Fall	+0.4	+0.1	+0.2
Winter	+0.5	+0.1	+0.2
Spring	+0.5	+0.2	+0.3

Use the seasonally corrected values for BI value and EPT richness to look up the scores for each in Table 3.

Add the BI and EPT Richness scores together and divide the result by two to get an average of the two. If the result does not fall midway between two integers (i.e. the result is not 1.5, 2.5, 3.5 or 4.5), round up or down to the nearest integer and assign the bioclassification as follows:

5 = Excellent 4 = Good 3 = Good-Fair 2 = Fair 1 = Poor

For those results that fall midway between integers, a "rounding decision" must be made based upon the average of the BI and EPT Richness scores (Avg Score) and EPT Abundance (EPT N). Use the values in Table 5 to determine the bioclassification.

Table 5. EPT N criteria for rounding decisions using Full Scale methods.

	Avg Score	EPT N		
		Mountain	Piedmont	Coastal Plain (CA)
Excellent	4.5	>= 191	>= 135	>= 108
Good	4.5	<= 190	<= 134	<= 107
Good	3.5	>= 125	>= 103	>= 91
Good-Fair	3.5	<= 124	<= 102	<= 90
Good-Fair	2.5	>= 85	>= 71	>= 46
Fair	2.5	<= 84	<= 70	<= 45
Fair	1.5	>= 45	>= 38	>= 18
Poor	1.5	<= 44	<= 37	<= 17

NOTE: The BAB benthos database will correctly report the bioclassification for summer Full Scale samples, as well as for non-summer samples if the number of seasonal EPT is entered.

Small Stream criteria

Small Stream criteria are appropriate to use when the following conditions are met:

- drainage area is less than or equal to 3.0 square miles
- site is located within either the mountain or piedmont ecoregion
- site is not within the Triassic Basins level IV ecoregion
- sample was collected during the months of April through June
- Qual-4 collection method was used

Use Table 6 to look up the bioclassification based upon the NCBI value for the sample and ecoregion from which the sampled was collected (there is no seasonal adjustment).

Table 6. NCBI thresholds for determining bioclassifications using Small Stream criteria.

	Mountain	**Piedmont**
Excellent	< 3.30	< 4.31
Good	3.30 – 4.73	4.31 - 5.18
Good-Fair	4.74 – 5.62	5.19 – 5.85
Fair	5.63 – 6.52	5.86 – 6.91
Poor	> 6.52	> 6.91

NOTE: The BAB benthos database will not give a correct bioclassification for streams meeting these criteria yet. The "Manual Assignment" box should be checked when entering data and the appropriate bioclassification (as determined above) entered to the drop-down box.

High-Quality Small Mountain Stream (HQSMS) criteria

High-Quality Small Mountain Stream (HQSMS) Criteria are appropriate to use when the following conditions are met:

- site is located in the mountain ecoregion
- drainage area is less than 3.5 square miles
- site is within an undisturbed drainage
- either EPT or Qual-4 collection method was used
- site cannot be evaluated using Small Stream criteria (above)

If the sample was collected outside of the June through September summer sampling period, make the seasonal adjustment as discussed under the use of EPT criteria earlier in this section.

Correction factors for HQSMS streams are 1.45 for sites with drainage areas less than 1.0 square miles and 1.25 for drainages between 1.0 and 3.5 square miles. Multiply EPT Richness (corrected for seasonality if necessary) by the appropriate correction factor to get the corrected EPT Richness, then look up that value in Table 1 on page 17 to determine the bioclassification for the site.

NOTE: The BAB benthos database will not give a correct bioclassification for streams meeting these criteria yet. The “Manual Assignment” box should be checked when entering data and the appropriate bioclassification (as determined above) entered to the drop-down box.

Unrated Small Streams

Apply these rules when the following conditions are met:

- drainage area is less than or equal to 3.0 square miles
- EPT or Qual-4 collection method was used
- site is located either in the mountains or piedmont OR normally flows during the entire year
- site is not within the Triassic Basins level IV ecoregion
- neither Small Stream nor HQSMS criteria can be applied

Look up the bioclassification from Table 1 on page 17 using the EPT Richness from the sample. If the result is either Excellent, Good, or Good-Fair, report as “Not Impaired”. Otherwise (if the result is Fair or Poor) report as “Not Rated”.

NOTE: Whether the collection method was EPT or Qual-4, enter as EPT to the database. The “Manual Assignment” box should be checked when entering data and the appropriate bioclassification (as determined above) entered to the drop-down box.

Coastal B (Boat) criteria

Coastal B criteria are appropriate to use when the following conditions are met:

- site is on an unwadeable freshwater river in the coastal plain
- site has little or no visible flow under normal or low conditions
- Boat collection method was used

A provisional bioclassification can be determined by looking up the EPT Richness for the sample in Table 7. The BAB benthos database will not give a correct provisional bioclassification. Also, the “Manual Assignment” box must be checked and “Not Rated” selected from the drop-down box.

Table 7. EPT Richness thresholds for determining provisional bioclassifications using Coastal B criteria.

	EPT S
Excellent	>11
Good	9-11
Good-Fair	6-8
Fair	3-5
Poor	< 3

Swamp Stream criteria

Swamp Stream criteria are appropriate to use when the following criteria are met:

- site is located on the coastal plain
- there is normally no flow at the site during a portion of the year (typically May-September)
- collection was made in February or the first fifteen days of March

Natural variability in the benthic invertebrate assemblages between regions of the coastal plain has been documented. Use the following key to determine the appropriate swamp region for the site:

1. Site is located in the level IV *Chesapeake-Pamlico Lowlands and Tidal Marshes* (63b) ecoregion and within HUC 03010205 (this hydrologic unit closely aligns with DWR's Pasquotank River Basin). Figure 3 ..... **Swamp Region C**
   Site is located elsewhere 2

2. Site located in level IV *Chesapeake-Pamlico Lowlands and Tidal Marshes* (63b) ecoregion or *Nonriverine Swamps and Peatlands* (63c) ecoregion AND in either the Tar or Neuse River Basins AND north of Neuse River ..... **Swamp Region D, CRITERIA NOT DEVELOPED, report bioclassification as "Not Rated"**
   Site is located elsewhere 3

3. Site is located in either the level IV *Mid-Atlantic Flatwoods* (63e) or *Mid-Atlantic Floodplains and Low Terraces* (63n) ecoregion AND the site is north of Neuse River. Figure 2 ..... **Swamp Region B**
   Site is located elsewhere 4

4. Site is located within the level IV *Atlantic Southern Loam Plains* (65l) or *Rolling Coastal Plain* (65m) ecoregions. Figure 1 ..... **Swamp Region A**
   Site is located elsewhere 5

5. Site is located within the level IV *Carolina Flatwoods* (63h) ecoregion AND with stream headwaters within the level IV *Nonriverine Swamps and Peatlands* (63c) ecoregion. Figures 4 through 6 ..... **Swamp Region P**
   Site is elsewhere 6

6. Site is located within the Waccamaw River drainage. Figure 7. ..... **Swamp Region S**
   Site does not meet any of the conditions above ..... **CRITERIA NOT DEVELOPED, report bioclassification as "Not Rated"**

To continue, scores for several metrics (corrected total taxa richness, NCBI value, corrected EPT richness, habitat) are determined. In general the BAB does not give a rating for stream sites where the pH is equal to 4.0 or less. There are also certain swamp regions for which sites are not rated when the pH is 4.5 or less (see below).

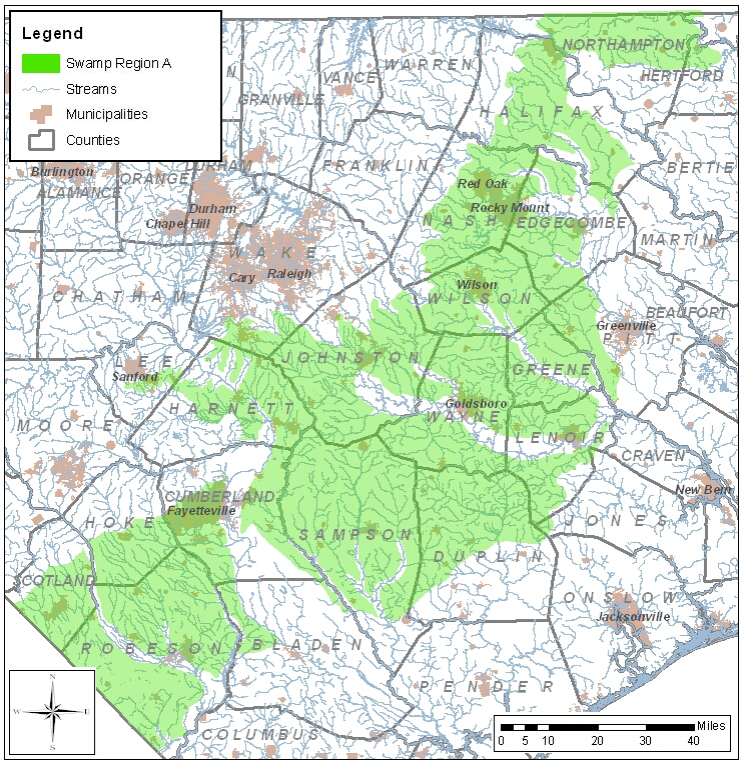

Figure 1. Swamp Region A.

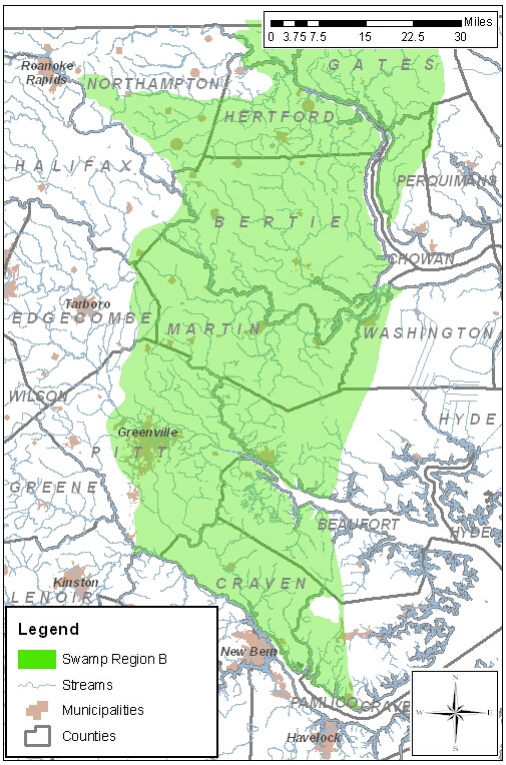

Figure 2. Swamp Region B.

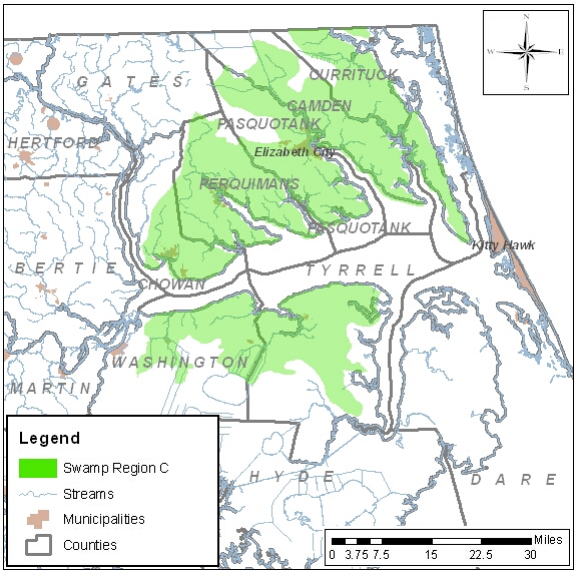

Figure 3. Swamp Region C.

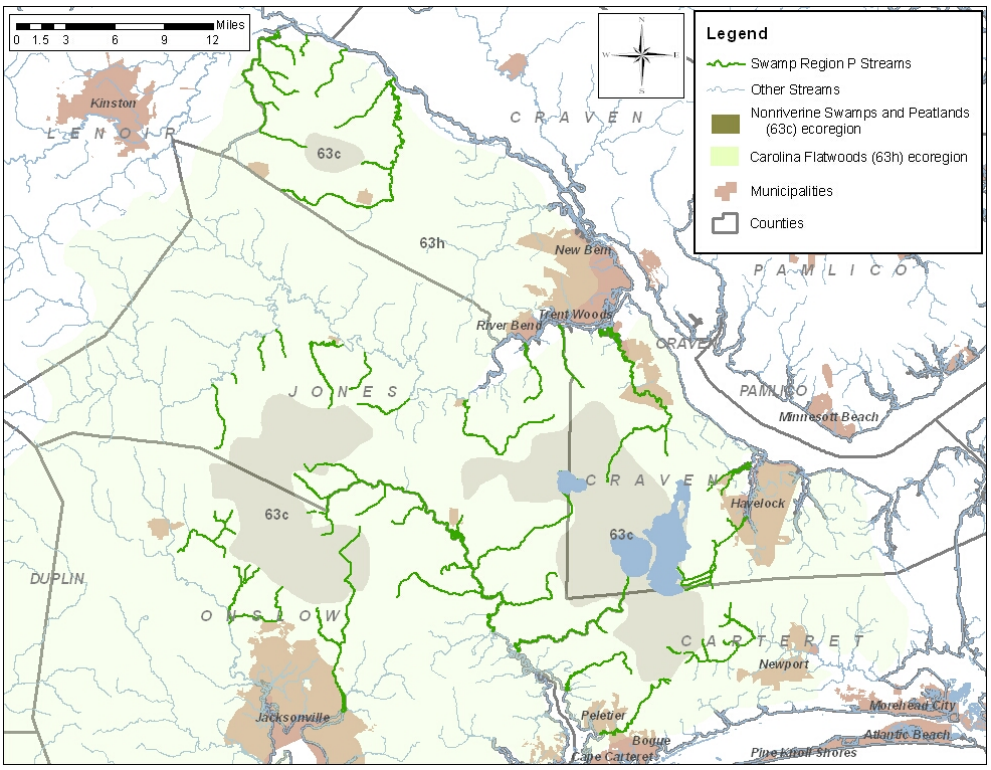

Figure 4. Northern portion of Swamp Region P.

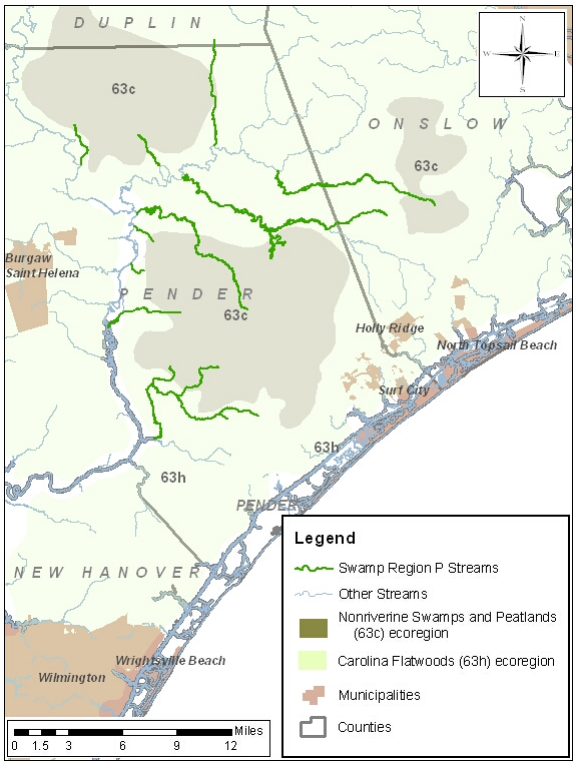

Figure 5. Middle portion of Swamp Region P.

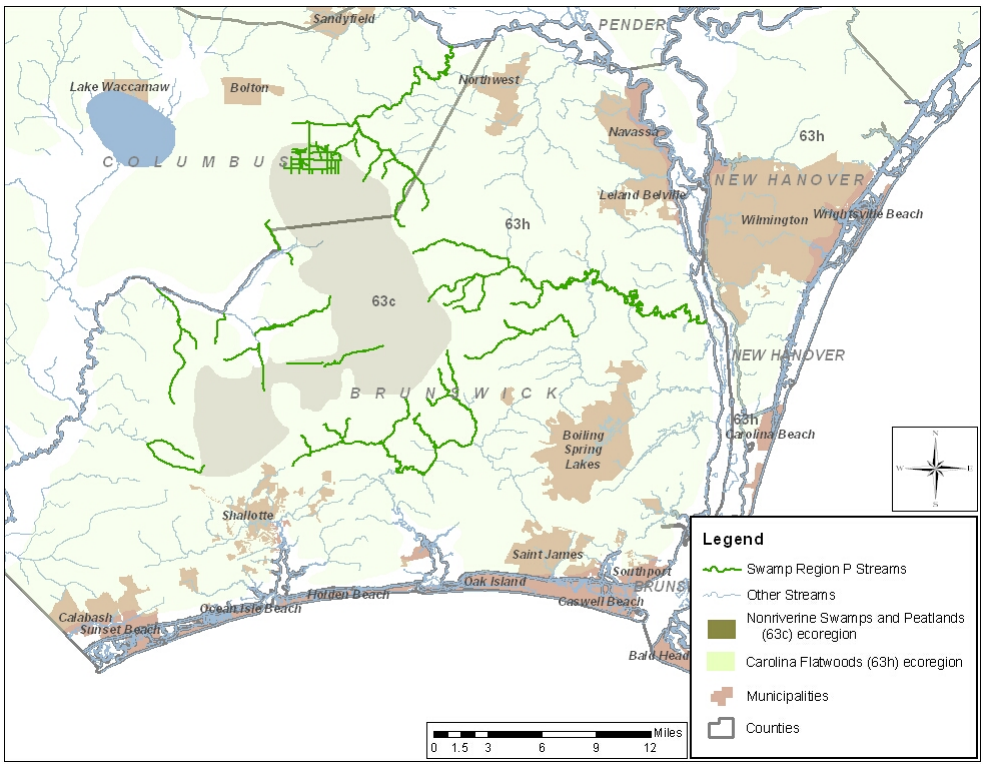

Figure 6. Southern portion of Swamp Region P.

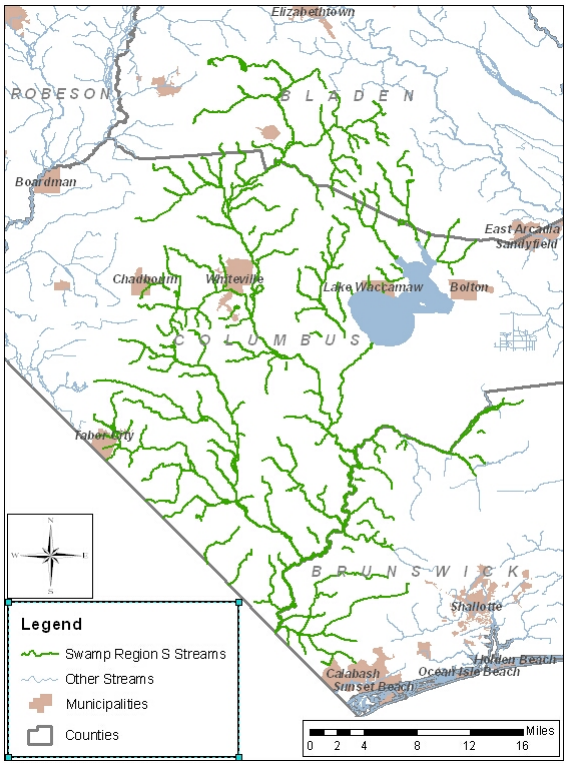

Figure 7. Swamp Region S.

Total Taxa Richness Score

To find the Total Taxa Richness Score for the site, first determine the Corrected Total Taxa Richness for the sample. **Corrected Total Taxa Richness is Total Taxa Richness + 8 for braided stream channels; Corrected Total Taxa Richness = Total Taxa Richness (no adjustment) for unbraided stream channels.**

The table used to look up the Taxa Richness Score depends upon swamp region. For regions A, P or S, use Table 8; for swamp region B use Table 9; for swamp region C use Table 10. For each table, find the row corresponding to the pH measured at the site in the leftmost column, and then find the column for that pH corresponding to the Corrected Total Taxa Richness. The number at the bottom of the column (5, 3, or 1) is the Taxa Richness Score.

Table 8. Taxa Richness Score lookup for Swamp Regions **A**, **P** or **S**.

pH	Corrected Total Taxa Richness		
≥5.5	>51	35-51	<35
5.4	>49	32-49	<32
5.3	>46	29-46	<29
5.2	>43	26-43	<26
5.1	>40	23-40	<23
5.0	>37	20-35	<20
4.9	>35	17-35	<17
4.8	>33	13-33	<13
4.7	>30	10-30	<10
4.6	>28	0-28	---
4.5	>26	0-26	---
4.4	>23	0-23	---
4.3	>20	0-20	---
4.2	>17	0-17	---
4.1	>14	0-14	---
≤4.0	Not Rated	Not Rated	Not Rated
Taxa Richness Score	5	3	1

Table 9. Taxa Richness Score lookup for Swamp Region **B**.

pH	Corrected Total Taxa Richness		
≥5.5	>38	25-38	<25
5.4	>36	23-36	<23
5.3	>34	21-34	<21
5.2	>32	19-32	<19
5.1	>30	17-30	<17
5.0	>28	≤28	---
4.9	>26	≤26	---
4.8	>24	≤24	---
4.7	>22	≤22	---
4.6	>20	≤20	---
4.5	>18	≤18	---
≤4.5	Not Rated	Not Rated	Not Rated
Taxa Richness Score	5	3	1

Table 10. Taxa Richness Score lookup for Swamp Region **C**.

pH	Corrected Total Taxa Richness		
≥4.1	>34	0-34	---
≤4.0	Not Rated	Not Rated	Not Rated
Taxa Richness Score	5	3	1

Biotic Index Score

Use Table 11 to determine the Biotic Index Score from the swamp region and the Biotic Index for the site.

Table 11. Biotic Index Score lookup for all swamp regions.

Swamp Region	Biotic Index Value		
A, P or S	<6.8	6.8-7.5	>7.5
B	<7.0	7.0-7.9	>7.9
C	<7.2	7.2-8.1	>8.1
Biotic Index Score	5	3	1

EPT Richness Score

To find the Total Taxa Richness Score for the site, first determine the Corrected EPT Richness for the sample. **Corrected EPT Richness is EPT Richness + 2 for braided stream channels; Corrected EPT Richness = EPT Richness (no adjustment) for unbraided stream channels.**

The table used to look up the EPT Richness Score depends upon swamp region. For regions A or P use Table 12; for swamp region S use Table 13; for swamp region B use Table 14. For each table, find the row corresponding to the pH measured at the site in the leftmost column, and then find the column for that pH corresponding to the Corrected EPT Richness. The number at the bottom of the column (5, 3, or 1) is the EPT Richness Score.

Swamp region C sites do not receive a score for EPT Richness.

Table 12. EPT Richness Score lookup for Swamp Regions **A** or **P**.

pH	Corrected EPT Richness		
>5.5	>17	7-17	0-6
5.4	>15	6-15	0-5
5.3	>13	5-13	0-4
5.2	>11	4-11	0-3
5.1	>9	3-9	0-2
5.0	>8	0-8	---
4.9	>7	0-7	---
4.8	>6	0-6	---
4.7	>5	0-5	---
4.6	>4	0-4	---
4.5	>4	---	---
≤4.5	Not Rated	Not Rated	Not Rated
EPT Richness Score	5	3	1

Table 13. EPT Richness Score lookup for Swamp Region **S**.

pH	Corrected EPT Richness		
≥4.1	>10	6-10	0-5
≤4.0	Not Rated	Not Rated	Not Rated
EPT Richness Score	5	3	1

Table 14. EPT Richness Score lookup for Swamp Region **B**.

pH	Corrected EPT Richness		
≥4.1	>5	2-4	0-1
≤4.0	Not Rated	Not Rated	Not Rated
EPT Richness Score	5	3	1

Habitat Score

Use Table 15 to determine the Habitat Score from the final value on the Habitat Assessment Field Data Sheet (which, unfortunately, is also known as the "Habitat Score") (Appendix 6).

Table 15. Habitat Score lookup for all swamp regions.

	Habitat Value		
	>79	60-79	<60
Habitat Score	5	3	1

Final Swamp Site Score and Bioclassification determination

Use one of the following formulas to get the final swamp site score.

Swamp Regions A, B, P, or S:

$$S = \frac{2\mathrm{B} + \mathrm{H} + \mathrm{E} + \mathrm{T} - 5}{2}$$

Swamp Region C:

$$S = \frac{2\mathrm{B} + \mathrm{H} + \mathrm{T}}{2}$$

where S = Site Score
B = Biotic Index Score
H = Habitat Score
E = EPT Score
T = Taxa Richness Score

Use Table 16 to determine the swamp bioclassification for the site using the Site Score from above.

NOTE: The BAB benthos database will not give a bioclassification for swamp sites; the “Manual Assignment” box must be checked and the correct classification ("Natural", "Moderate", "Severe", “Not Rated”) selected from the drop-down box.

Table 16. Swamp Bioclassification lookup.

	Final Swamp Site Score		
	9-10	4-8	1-3
Swamp Bioclassification	Natural	Moderate	Severe

Midge Deformity Analysis

When a discharge contains both organics and toxic chemicals, the resulting community is often dominated by typical organic indicator species, especially *Chironomus* larvae. Under conditions of organic loading (low dissolved oxygen, high BOD), it would be useful to deduce the presence or absence of toxic chemicals. Researchers have shown that deformities in chironomid larvae (especially Chironomus) are associated with contaminated sediments. Using larvae from old samples and toxicity information from the DWR Aquatic Toxicology Branch, a good correlation was found between toxicity and *Chironomus* mentum deformities, leading to the use of analysis of these deformities as a screening tool for toxicity. At least 20-25 *Chironomus* heads should be slide mounted from any site to be screened.

Deformities are classified into three groups:

Class I: Slight deformities which are difficult to separate from "chipped" teeth.
Class II. Clear deformities, including extra teeth, missing teeth, large gaps, and distinct asymmetry.
Class III. Severe deformation which includes at least two Class II characters.

A "Toxic Score" is computed for each site which gives greater weight to more severe deformities:

$$\frac{[\#\text{ Class I} + 2(\#\text{ Class II}) + 3(\#\text{ Class III})] \times 100}{\text{Total \# larvae}}$$

No significant between-group differences were found for Excellent, Good and Good-Fair nontoxic sites. The percent deformities for these unpolluted sites averaged about 5%, with a mean toxic score of about 7. Fair and Poor nontoxic sites are combined into a polluted/nontoxic group, with a deformity rate of 12% and a mean toxic score of 18. "Nontoxic" conditions for this group includes solely organic dischargers (animal wastes) and natural organic loading (swamps). A Fair/Toxic group had a 25% deformity rate and a mean toxic score of 52. A further significant increase was seen for the Poor/Toxic group: mean deformity rate = 45%, mean toxic score = 100. Both toxic groups also are characterized by a high proportion of Class II and Class III deformities.

QUALITY ASSURANCE

Quality assurance begins with following the procedures found in this manual, or documenting any changes in methods. It includes taking proper care of equipment, looking for holes in nets before sampling, and rinsing all nets and tubs carefully between sites. All meters must be calibrated prior to sampling; calibrations must be checked at the end of each sampling day. Quality assurance of field sampling also includes annual "overlap" samples to determine that reproducible results are being attained. Overlap samples consist of two separate collections by different teams at the same site and within 2-3 weeks, with no appreciable rains in between. In addition, field crews typically are not made up of the same three benthic biologists.

Taxonomic quality control in the laboratory is maintained in several ways. Organisms are first identified using current, regional identification manuals and other appropriate taxonomic literature. If questions occur, identifications are verified by other taxonomists in the Biological Assessment Branch. In order to maintain consistency in the taxonomic identifications, four Benthos Taxonomy Documents[1] have been compiled, one each for the EPT and Coleoptera orders. These documents specify the level of identification to be used (genus or species), the references to be used for the IDs, and any pertinent ecological or distribution data available. These documents will be updated regularly and other orders added as resources allow. Copies of all taxonomic papers used have been placed in a readily accessible location in the laboratory for the use of all benthic biologists. Taxonomic assistance is obtained from specialists when appropriate. Reference specimens (most verified by taxonomic experts) are maintained in a reference cabinet, and samples are stored for future reference. A reference specimen list is maintained and updated periodically.

Checks are performed on ten percent of the samples identified by BAB biologists. When each biologist completes a block of ten samples, one of the ten is randomly selected for re-identification by a second, randomly selected biologist (the "QA biologist"). Random selection of samples and QA biologists is performed by a computer program at the beginning of each month for all BAB invertebrate biologists. The randomly selected sample (the "QA sample") is given to the randomly selected QA biologist for re-identification, which should be completed within four weeks. After QA discussions (which will involve other biologists) the BAB supervisor scores the original identification for accuracy (see Table 17) and logs the information into a QA database.

If a sample fails a QA test, three additional samples are randomly selected from the nine remaining samples in the QA block and checked by other BAB biologists. If all three samples pass, no further action is taken. If any one of the three samples fails QA, the remaining six samples from the block are also reviewed by the other biologists in the branch.

Scoring the original identifications of QA samples involves taxonomic identification, abundance categories, and data entry. Error points are assigned in accordance with Table 17. The final score is the sum of error points divided by the number of taxa in the sample (as determined by the QA biologist) then multiplied by 100. A score of less than 90 is a fail.

[1] available here: http://portal.ncdenr.org/web/wq/taxonmanual

Table 17. Errors and associated points for taxonomic quality assurance checks. "Correct identification" is a taxonomic identification agreed upon by two or more BAB taxonomists.

Error Description	Point Deduction Per Error	Error Example
Misidentification	1	*Baetis pluto* is the correct identification. The original taxonomist misidentified the specimen as *Baetis flavistriga*.
Phantom Identification	1	Original taxonomist identified a specimen as *Baetis pluto*. However, no such specimen was found by the QA taxonomist. Original taxonomist is allowed the option of searching the sample for the specimen.
Inverse Phantom Identification	1	Original taxonomist failed to find *Baetis pluto*. However the QA taxonomist found that taxon.
Transcription	1	Specimen correctly identified but not included on bench sheet. For example, original taxonomist mounts maxillary crown of *Stenacron interpunctatum* (headless nymph found in sample) but taxon not recorded on the benchsheet.
Insufficient Effort	0.5	*Baetis pluto* is the correct identification. The original taxonomist believed the specimen too small, or too damaged, or that required morphological characters were absent or indiscernible and therefore left the specimen at *Baetis* sp. Original taxonomist is allowed to contest.
Over Effort	0.5	*Baetis* sp. is the correct identification. The original taxonomist identified the specimen as *Baetis flavistriga* despite the fact that the specimen was too small, or too damaged, or that required morphological characters were absent or indiscernible. Original taxonomist is allowed to contest.
Abundance Classification	0.25 for each level of increase or decrease	Original taxonomist entered *Baetis pluto* as Rare. However, the QA taxonomist counted ten or more *Baetis pluto* so the abundance class should have been Abundant. The total point error in this instance would be 0.50.

REFERENCES FOR BENTHIC MACROINVERTEBRATES

Benke, A.C., D.M. Gillespie, & T.C. Van Arsdall. 1984. Invertebrate productivity in a subtropical blackwater river: the importance of habitat and life history. Ecological Monographs 54:25-63.

Bode, R.W. and K.W. Simpson. 1982. Communities in large lotic systems: impacted vs. unimpacted. Abstract, Thirtieth Annual Meeting, North American Benthological Society.

Burton, G.A. Jr. 1991. Assessing the toxicity of freshwater sediments. Environmental Toxicology and Chemistry. 10: 1585-1627.

Clements, W.H. 1994. Benthic invertebrate community response to heavy metals in the Upper Arkansas River basin, Colorado. JNABS 13:30-44.

Cranston, P.S. 1990. Biomonitoring and invertebrate taxonomy. Environmental Monitoring and Assessment 14: 265-273.

Eaton, L. E. & D. R. Lenat. 1991. Comparison of a rapid bioassessment method with North Carolina's qualitative macroinvertebrate collection method. Journal of the North American Benthological Society 10:335-338.

Engel, S.R. & J.R. Voshell, Jr. 2002. Volunteer Biological Monitoring: Can it accurately assess the ecological condition of streams? American Entomologist 48 (3): 164-177.

Griffith, G.E., J.M. Omernik, J.A. Comstock, M.P. Shafale, D.R. Lenat, T. MacPherson, J.B. Glover, W.H. McNab, and V.B. Shelburne. 2002. Ecoregions of North and South Carolina. (2 sided color poster with map, descriptive text, summary tables, and photographs). U.S. Geological Survey, Reston, VA. Scale 1:1,500,000.

Hilsenhoff, W.L. 1987. An improved biotic index of organic stream pollution. Great lakes Entomologist 20: 31-39.

Larsen, D. P. and A.T. Herlihy. 1998. The dilemma of sampling streams for macroinvertebrate richness. JNABS 17: 359-366.

Lenat, D.R. and V.H. Resh. 2001. Taxonomy and stream ecology - The benefits of genus and species-level identifications. Journal of the North American Benthological Society, in press.

Lenat, D.R. 1993. A biotic index for the southeastern United States: derivation and list of tolerance values, with criteria for assigning water quality ratings. JNABS 12: 279-290.

Lenat, D.R. 1988. Water quality assessment of streams using a qualitative collection method for benthic macroinvertebrates. Journal of the North American Benthological Society 7: 222-233.

Neuswanger, D.J., W.W. Taylor and J.B. Regnolds. 1982. Comparison of macroinvertebrate herptobenthos and haptobenthos in side channel and slough in the Upper Mississippi River. Freshwat. Invertebr. Biol. 1(3):13-24.

NCDWQ, 2009. Biocriteria for the Small Streams of the North Carolina Mountains and Piedmont: Memorandum. NC Dept. of Environment and Natural Resources, Division of Water Quality. May 29, 2009.

Resh, V.H. and J.D. Unzicker. 1975. Water quality monitoring and aquatic organisms: the importance of species identification. J. Water Poll. Control Fed. 47:9-19.

Rosenberg, D. M., H. V. Danks, and D. M. Lehmkuhl. 1986. Importance of insects in environmental impact assessment. Environmental Management 10: 773-783.

USEPA, 2000. Stressor Identification Guidance Document. Office of Water & Office of Research & Development. EPA/822/B-00/025

Waters, Thomas F. Sediment in Streams: Sources, Biological Effects and Controls. 1995. American Fisheries Society Monograph 7.

Vannote, R.L., G.W. Minshall, K.W. Cummins, J.R. Sedell & C.E. Cushing. 1980. The river continuum concept. Canadian J. of Fisheries & Aquatic Sciences 37:130-137.

Appendix 1. Tolerance Values for Benthic Macroinvertebrates Used in NCBI.
Taxa collected fewer than 50 times have not been assigned a TV and are not used in the NCBI. Taxa higher than genus also have not been assigned a TV. Tolerance values were last updated in April 2010.

Order	Family	Latin Name	Tolerance Value
Ephemeroptera	Ameletidae	Ameletus lineatus	2.4
	Baetidae	Acentrella alachua	3.0
		Acentrella nadineae	1.9
		Acentrella parvula	4.8
		Acentrella spp	2.5
		Acentrella turbida	2.0
		Acerpenna pygmaea	3.7
		Baetis flavistriga	6.8
		Baetis intercalaris	5.0
		Baetis pluto	3.4
		Baetis tricaudatus	1.5
		Callibaetis spp	9.2
		Centroptilum spp	3.8
		Cloeon spp	7.3
		Diphetor hageni	1.1
		Heterocloeon amplum	3.4
		Heterocloeon curiosum	2.1
		Heterocloeon spp	3.7
		Iswaeon anoka	4.4
		Paracloeodes spp	8.0
		Plauditus cestus	4.6
		Plauditus dubius gr	2.2
		Procloeon spp	1.9
		Pseudocloeon ephippiatum	3.5
		Pseudocloeon frondale	4.6
		Pseudocloeon propinquum	5.8
	Baetiscidae	Baetisca berneri	1.4
		Baetisca carolina	4.2
		Baetisca spp	3.2
	Caenidae	Brachycercus spp	2.1
		Caenis spp	6.8
	Ephemerellidae	Attenella attenuata	1.1
		Dannella simplex	3.4
		Drunella allegheniensis	0.3
		Drunella conestee	0.0
		Drunella cornutella	0.0
		Drunella lata	0.0
		Drunella tuberculata	0.0
		Drunella walkeri	0.6
		Drunella wayah	0.0
		Ephemerella catawba	0.0
		Ephemerella catawba/dorothea	4.0
		Ephemerella dorothea	3.3
		Ephemerella hispida	0.1
		Ephemerella invaria	2.6
		Ephemerella rossi gr	0.0
		Ephemerella rotunda	1.8
		Ephemerella spp	2.1
		Eurylophella bicolor	4.8
		Eurylophella doris	7.0

Appendix 1. Tolerance Values for Benthic Macroinvertebrates Used in NCBI.
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Order	Family	Latin Name	Tolerance Value
Ephemeroptera	Ephemerellidae	Eurylophella funeralis	2.5
		Eurylophella spp	4.0
		Eurylophella temporalis gr	4.8
		Eurylophella verisimilis	3.9
		Penelomax septentrionalis	2.1
		Serratella carolina	0.0
		Serratella serrata	1.4
		Serratella serratoides	1.7
		Telagonopsis deficiens	2.6
	Ephemeridae	Ephemera blanda	2.4
		Ephemera guttalata	0.0
		Ephemera spp	2.0
		Hexagenia spp	4.4
	Heptageniidae	Cinygmula subaequalis	0.0
		Epeorus dispar	1.0
		Epeorus pleuralis	1.5
		Epeorus spp	1.6
		Epeorus vitreus	1.2
		Heptagenia marginalis	2.2
		Heptagenia pulla	2.2
		Heptagenia spp	1.9
		Leucrocuta aphrodite	2.9
		Leucrocuta spp	2.0
		Maccaffertium carlsoni	2.1
		Maccaffertium exiguum	3.8
		Maccaffertium ithaca	3.0
		Maccaffertium lenati	2.5
		Maccaffertium mediopunctatum	4.2
		Maccaffertium meririvulanum	0.5
		Maccaffertium mexicanum	4.7
		Maccaffertium modestum	5.7
		Maccaffertium pudicum	2.1
		Maccaffertium terminatum	4.4
		Maccaffertium vicarium	1.5
		Rhithrogena exilis	0.0
		Rhithrogena spp	0.0
		Rhithrogena uhari	0.0
		Stenacron carolina	1.3
		Stenacron interpunctatum	6.4
		Stenacron pallidum	2.8
		Stenonema femoratum	6.9
	Isonychiidae	Isonychia spp	3.6
	Leptohyphidae	Tricorythodes spp	5.0
	Leptophlebiidae	Habrophlebia vibrans	0.3
		Leptophlebia spp	6.0
		Paraleptophlebia spp	1.2
	Neoephemeridae	Neoephemera purpurea	1.5
	Polymitarcyidae	Ephoron leukon	1.5
	Potamanthidae	Anthopotamus distinctus	1.6
		Anthopotamus spp	1.5
	Siphlonuridae	Siphlonurus spp	6.0
Plecoptera	Capniidae	Allocapnia spp	3.3
	Chloroperlidae	Alloperla spp	1.0

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Order	Family	Latin Name	Tolerance Value
Plecoptera	Chloroperlidae	Haploperla brevis	1.4
		Suwallia marginata	2.6
		Sweltsa spp	0.2
	Leuctridae	Leuctra spp	1.5
	Nemouridae	Amphinemura spp	3.8
		Prostoia spp	5.2
	Peltoperlidae	Tallaperla spp	1.3
	Perlidae	Acroneuria abnormis	2.1
		Acroneuria arenosa	2.4
		Acroneuria carolinensis	1.2
		Acroneuria evoluta	1.7
		Acroneuria lycorias	2.1
		Agnetina spp	1.1
		Beloneuria spp	0.0
		Eccoptura xanthenes	4.7
		Neoperla spp	2.1
		Paragnetina fumosa	3.6
		Paragnetina ichusa/media	0.2
		Paragnetina immarginata	1.1
		Paragnetina kansensis	1.9
		Perlesta spp	2.9
		Perlinella drymo	1.3
	Perlodidae	Clioperla clio	5.2
		Cultus decisus complex	1.5
		Diploperla duplicata	2.8
		Helopicus subvarians	1.2
		Isoperla bilineata gr	5.2
		Isoperla holochlora	0.7
		Isoperla nr holochlora	0.0
		Isoperla nr namata	2.5
		Isoperla orata	0.0
		Isoperla similis gr	0.8
		Isoperla sp A	1.2
		Isoperla spp	3.2
		Isoperla transmarina gr	4.8
		Malirekus hastatus	1.0
		Remenus bilobatus	0.9
	Pteronarcyidae	Pteronarcys biloba	0.0
		Pteronarcys dorsata	2.4
		Pteronarcys proteus	0.4
		Pteronarcys spp	1.8
	Taeniopterygidae	Strophopteryx spp	3.3
		Taeniopteryx burksi	6.6
		Taeniopteryx spp	6.0
Trichoptera	Apataniidae	Apatania spp	0.6
	Brachycentridae	Brachycentrus appalachia	1.0
		Brachycentrus lateralis	1.9
		Brachycentrus nigrosoma	3.1
		Brachycentrus numerosus	1.7
		Brachycentrus spinae	0.0
		Brachycentrus spp	2.2
		Micrasema bennetti	0.0
		Micrasema charonis	1.0

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Order	Family	Latin Name	Tolerance Value
Trichoptera	Brachycentridae	Micrasema rickeri	0.0
		Micrasema wataga	2.2
	Calamoceratidae	Anisocentropus pyraloides	1.3
		Heteroplectron americanum	2.0
	Dipseudopsidae	Phylocentropus spp	4.8
	Glossosomatidae	Agapetus spp	0.0
		Glossosoma spp	1.4
		Protoptila spp	2.3
	Goeridae	Goera calcarata	1.0
		Goera spp	0.7
	Helicopsychidae	Helicopsyche borealis	0.0
	Hydropsychidae	Arctopsyche irrorata	0.0
		Ceratopsyche alhedra	0.0
		Ceratopsyche bronta	2.3
		Ceratopsyche macleodi	0.7
		Ceratopsyche morosa	2.3
		Ceratopsyche slossonae	0.0
		Ceratopsyche sparna	2.5
		Cheumatopsyche spp	6.6
		Diplectrona modesta	2.3
		Hydropsyche betteni	7.9
		Hydropsyche decalda	3.2
		Hydropsyche demora	2.6
		Hydropsyche incommoda	4.6
		Hydropsyche phalerata	3.7
		Hydropsyche rossi	4.8
		Hydropsyche scalaris	2.6
		Hydropsyche venularis	5.1
		Macrostemum spp	3.4
		Parapsyche cardis	0.0
	Hydroptilidae	Hydroptila spp	6.5
		Leucotrichia pictipes	4.6
	Lepidostomatidae	Lepidostoma spp	1.0
	Leptoceridae	Ceraclea ancylus	2.8
		Ceraclea maculata	6.2
		Ceraclea spp	2.2
		Ceraclea transversa	2.8
		Mystacides sepulchralis	2.6
		Nectopsyche candida	6.5
		Nectopsyche exquisita	4.3
		Nectopsyche pavida	3.9
		Oecetis georgia	3.6
		Oecetis nocturna	5.0
		Oecetis persimilis	4.6
		Oecetis scala gr	2.7
		Oecetis spp	5.1
		Setodes spp	0.0
		Triaenodes ignitus	4.8
		Triaenodes injustus	2.7
		Triaenodes perna/helo	3.8
		Triaenodes spp	4.1
	Limnephilidae	Hydatophylax argus	2.4
		Ironoquia punctatissima	6.7

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Order	Family	Latin Name	Tolerance Value
Trichoptera	Limnephilidae	Pycnopsyche gentilis	1.8
		Pycnopsyche guttifer	2.2
		Pycnopsyche lepida gr	3.9
		Pycnopsyche scabripennis	2.5
		Pycnopsyche spp	2.5
	Molannidae	Molanna blenda	1.6
		Molanna tryphena	2.4
	Odontoceridae	Psilotreta spp	0.5
	Philopotamidae	Chimarra spp	3.3
		Dolophilodes spp	1.0
		Wormaldia spp	2.4
	Phryganeidae	Oligostomis pardalis	6.2
		Ptilostomis spp	5.9
	Polycentropodidae	Cyrnellus fraternus	6.8
		Neureclipsis spp	4.0
		Nyctiophylax celta	0.7
		Nyctiophylax moestus	3.8
		Nyctiophylax nephophilus	0.6
		Nyctiophylax spp	0.8
		Polycentropus spp	3.1
	Psychomyiidae	Lype diversa	3.9
		Psychomyia flavida	3.0
		Psychomyia nomada	2.0
	Rhyacophilidae	Rhyacophila acutiloba	0.0
		Rhyacophila atrata	0.0
		Rhyacophila carolina	0.4
		Rhyacophila fenestra/ledra	4.6
		Rhyacophila formosa	0.1
		Rhyacophila fuscula	1.6
		Rhyacophila nigrita	0.0
		Rhyacophila torva	1.5
	Sericostomatidae	Fattigia pele	0.0
	Uenoidae	Neophylax consimilis	0.3
		Neophylax fuscus	0.0
		Neophylax mitchelli	0.0
		Neophylax oligius	2.4
		Neophylax ornatus	1.3
		Neophylax spp	1.6
Odonata	Aeshnidae	Basiaeschna janata	7.1
		Boyeria grafiana	3.8
		Boyeria vinosa	5.8
		Nasiaeschna pentacantha	6.6
	Calopterygidae	Calopteryx spp	7.5
		Hetaerina spp	4.9
	Coenagrionidae	Argia spp	8.3
		Enallagma spp	8.5
		Ischnura spp	9.5
	Cordulegasteridae	Cordulegaster spp	5.7
	Corduliidae	Epicordulia princeps	7.3
		Helocordulia spp	5.8
		Neurocordulia obsoleta	5.3
		Neurocordulia spp	5.3
		Neurocordulia virginiensis	1.1

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Order	Family	Latin Name	Tolerance Value
Odonata	Corduliidae	Somatochlora spp	8.9
		Tetragoneuria spp	8.0
	Gomphidae	Dromogomphus spp	5.6
		Gomphus spiniceps	6.1
		Gomphus spp	5.9
		Hagenius brevistylus	4.4
		Lanthus parvulus	0.6
		Lanthus spp	1.6
		Lanthus vernalis	0.8
		Ophiogomphus spp	5.9
		Progomphus obscurus	8.2
		Stylogomphus albistylus	5.0
	Libellulidae	Libellula spp	9.4
		Pachydiplax longipennis	9.6
		Perithemis spp	9.4
		Plathemis lydia	9.8
	Macromiidae	Macromia spp	6.2
Hemiptera	Belostomatidae	Belostoma spp	9.5
	Corixidae	Sigara spp	8.7
	Nepidae	Ranatra spp	6.3
Megaloptera	Corydalidae	Corydalus cornutus	5.2
		Nigronia fasciatus	6.1
		Nigronia serricornis	4.6
	Sialidae	Sialis spp	7.0
Coleoptera	Dryopidae	Helichus basalis	0.5
		Helichus lithophilus	3.0
		Helichus spp	4.1
	Dytiscidae	Coptotomus spp	8.5
		Hydroporus spp	7.0
		Laccophilus spp	9.8
		Lioporeus spp	4.0
		Neoporus mellitus	3.9
		Neoporus spp	5.0
		Stictotarsus griseostriatus	4.9
	Elmidae	Ancyronyx variegatus	6.8
		Dubiraphia spp	5.5
		Dubiraphia vittata	5.0
		Macronychus glabratus	4.7
		Microcylloepus pusillus	3.3
		Optioservus ovalis	2.1
		Optioservus spp	2.1
		Oulimnius latiusculus	1.9
		Promoresia elegans	2.1
		Promoresia spp	3.1
		Promoresia tardella	0.0
		Stenelmis crenata	7.8
		Stenelmis spp	5.6
	Gyrinidae	Dineutus spp	5.0
		Gyrinus spp	5.8
	Haliplidae	Peltodytes spp	8.4
	Hydrophilidae	Berosus spp	8.8
		Enochrus spp	8.5
		Laccobius spp	6.5

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Order	Family	Latin Name	Tolerance Value
Coleoptera	Hydrophilidae	Sperchopsis tessellatus	4.4
		Tropisternus spp	9.3
	Psephenidae	Ectopria nervosa	4.3
		Psephenus herricki	2.3
	Ptilodactylidae	Anchytarsus bicolor	2.4
Diptera	Blephariceridae	Blepharicera spp	0.0
	Ceratopogonidae	Atrichopogon spp	6.1
		Culicoides spp	8.6
		Palpomyia complex	5.7
	Chironomidae	Ablabesmyia mallochi	7.4
		Ablabesmyia rhamphe gr	6.8
		Brillia flavifrons	3.9
		Brillia spp	5.7
		Brundiniella eumorpha	2.0
		Cardiocladius spp	6.2
		Chironomus spp	9.3
		Cladotanytarsus cf daviesi	2.8
		Cladotanytarsus sp B	4.7
		Cladotanytarsus spp	4.0
		Clinotanypus spp	7.8
		Corynoneura spp	5.7
		Cricotopus annulator complex	8.4
		Cricotopus bicinctus	8.7
		Cricotopus fugax	5.6
		Cricotopus infuscatus gr	9.1
		Cricotopus vierriensis gr	5.4
		Cryptochironomus blarina gr	8.5
		Cryptochironomus fulvus	6.7
		Cryptochironomus spp	6.4
		Cryptotendipes spp	6.2
		Demicryptochironomus spp	2.2
		Diamesa spp	6.6
		Dicrotendipes fumidus	8.8
		Dicrotendipes modestus	9.4
		Dicrotendipes neomodestus	7.9
		Dicrotendipes nervosus	9.5
		Dicrotendipes simpsoni	9.8
		Dicrotendipes spp	7.2
		Diplocladius cultriger	8.0
		Eukiefferiella brehmi gr	2.5
		Eukiefferiella brevicalcar gr	2.9
		Eukiefferiella claripennis gr	6.2
		Eukiefferiella devonica gr	3.4
		Eukiefferiella gracei gr	4.4
		Eukiefferiella pseudomontana gr	1.3
		Glyptotendipes spp	8.6
		Heleniella spp	0.0
		Hydrobaenus spp	9.2
		Labrundinia pilosella	6.2
		Labrundinia spp	6.2
		Larsia spp	6.5
		Lopescladius spp	1.2
		Micropsectra spp	2.4

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Order	Family	Latin Name	Tolerance Value
Diptera	Chironomidae	Microtendipes pedellus gr	3.9
		Microtendipes rydalensis gr	1.1
		Microtendipes spp	4.6
		Nanocladius downesi	2.4
		Nanocladius spp	7.4
		Natarsia spp	9.6
		Nilotanypus fimbriatus	4.9
		Nilotanypus spp	4.1
		Nilothauma spp	5.1
		Odontomesa fulva	4.9
		Orthocladius clarkei gr	5.6
		Orthocladius dorenus	5.8
		Orthocladius dubitatus	9.0
		Orthocladius lignicola	5.4
		Orthocladius luteipes/thienemanni	6.3
		Orthocladius nigritus	3.8
		Orthocladius obumbratus gr	8.1
		Orthocladius robacki	6.4
		Orthocladius spp	4.4
		Pagastia orthogonia	1.5
		Parachaetocladius abnobaeus	0.7
		Parachironomus spp	8.0
		Paracladopelma spp	6.3
		Paracladopelma undine	4.5
		Parakiefferiella sp A	8.5
		Parakiefferiella spp	4.8
		Paralauterborniella nigrohalteralis	4.9
		Paramerina spp	4.1
		Parametriocnemus spp	3.9
		Paratanytarsus spp	8.0
		Paratendipes spp	5.6
		Pentaneura inconspicua	5.0
		Phaenopsectra obediens gr	6.6
		Phaenopsectra punctipes gr	7.1
		Polypedilum aviceps	3.6
		Polypedilum fallax/sp A	6.5
		Polypedilum flavum	5.7
		Polypedilum halterale gr	7.4
		Polypedilum illinoense gr	8.7
		Polypedilum laetum	2.2
		Polypedilum scalaenum gr	8.5
		Potthastia cf gaedii	2.4
		Potthastia longimana	8.4
		Procladius spp	8.8
		Prodiamesa olivacea	8.8
		Psectrotanypus dyari	10.0
		Pseudochironomus spp	4.9
		Rheocricotopus robacki	7.9
		Rheocricotopus spp	4.7
		Rheocricotopus tuberculatus	4.7
		Rheopelopia spp	0.3
		Rheosmittia spp	6.8
		Rheotanytarsus spp	6.5

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Order	Family	Latin Name	Tolerance Value
Diptera	Chironomidae	Robackia claviger	1.9
		Robackia demeijerei	4.3
		Saetheria tylus	7.3
		Stelechomyia perpulchra	4.0
		Stempellinella spp	5.6
		Stenochironomus spp	6.3
		Stictochironomus spp	5.4
		Sublettea coffmani	1.4
		Sympotthastia spp	4.5
		Synorthocladius spp	4.2
		Tanytarsus sp 2	6.9
		Tanytarsus sp 3	7.3
		Tanytarsus sp 4	4.7
		Tanytarsus sp 6	7.8
		Tanytarsus sp A	6.9
		Tanytarsus sp C	6.1
		Tanytarsus sp L	4.7
		Tanytarsus sp M	3.2
		Tanytarsus sp P	4.8
		Tanytarsus sp U	6.4
		Tanytarsus spp	6.6
		Thienemaniella spp	6.4
		Thienemaniella xena	8.0
		Thienemannimyia gr	8.4
		Tribelos jucundum	5.7
		Tribelos spp	6.4
		Tvetenia bavarica gr	3.6
		Tvetenia vitracies	3.5
		Xenochironomus xenolabis	6.6
		Xylotopus par	6.1
		Zavrelia spp	6.1
		Zavrelimyia spp	8.6
	Culicidae	Anopheles spp	8.6
	Dixidae	Dixa spp	2.5
		Dixella indiana	4.9
	Rhagionidae	Atherix lantha	1.8
		Atherix spp	0.9
	Simuliidae	Prosimulium mixtum	3.6
		Prosimulium spp	4.5
		Simulium spp	4.9
		Simulium venustum	7.3
		Simulium vittatum	9.1
	Tabanidae	Chrysops spp	6.7
		Tabanus spp	8.5
	Tanyderidae	Protoplasa fitchii	4.0
	Tipulidae	Antocha spp	4.4
		Dicranota spp	0.0
		Hexatoma spp	3.5
		Limonia spp	9.3
		Polymeda/Ormosia spp	6.5
		Pseudolimnophila spp	6.2
		Tipula spp	7.5

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Order	Family	Latin Name	Tolerance Value
Oligochaeta	Naididae	Dero spp	9.8
		Nais spp	8.7
		Pristina spp	7.7
		Slavina appendiculata	8.4
		Stylaria lacustris	8.4
	Tubificidae	Aulodrilus pluriseta	5.6
		Branchiura sowerbyi	8.6
		Ilyodrilus templetoni	9.3
		Limnodrilus hoffmeisteri	9.4
		Limnodrilus spp	8.5
		Spirosperma nikolskyi	6.0
		Tubifex tubifex	9.9
Gastropoda	Ancylidae	Ferrissia spp	6.6
		Laevapex fuscus	6.6
	Hydrobiidae	Amnicola spp	4.1
	Lymnaeidae	Pseudosuccinea columella	7.7
		Stagnicola spp	8.1
	Physidae	Physa spp	8.7
	Planorbidae	Helisoma anceps	6.6
		Micromenetus dilatatus	7.6
	Pleuroceridae	Elimia spp	2.7
		Leptoxis spp	1.7
	Viviparidae	Campeloma decisum	5.8
Bivalvia	Corbiculidae	Corbicula fluminea	6.6
	Sphaeriidae	Pisidium spp	6.6
		Sphaerium spp	7.2
	Unionidae	Elliptio complanata	4.7
		Elliptio spp	4.9
Crustacea	Asellidae	Caecidotea spp	8.4
		Lirceus spp	7.4
	Cambaridae	Cambarus (P.) sp C	6.3
		Cambarus spp	7.5
		Orconectes spp	2.7
		Procambarus spp	9.3
	Gammaridae	Crangonyx spp	7.2
		Gammarus fasciatus	7.0
		Gammarus spp	7.1
	Palaemonidae	Palaemonetes paludosus	6.1
		Palaemonetes spp	8.7
	Talitridae	Hyalella spp	7.2
Other	Erpobdellidae	Erpobdella/Mooreobdella spp	8.6
		Mooreobdella tetragon	9.4
	Glossiphoniidae	Desserobdella phalera	6.6
		Gloiobdella elongata	9.1
		Helobdella triserialis	9.3
		Placobdella papillifera	8.2
		Placobdella parasitica	8.9
	Planariidae	Cura foremanii	5.5
		Dugesia tigrina	7.1
	Pyralidae	Petrophila spp	3.6
	Sisyridae	Climacia areolaris	6.5
	Tetrastemmatidae	Prostoma graecens	6.6

Appendix 2. Benthic Macroinvertebrate Field and Lab Equipment

A. Field Equipment

Kick nets
Sweep nets
Sand bag sampler
Fine-mesh samplers
Petite Ponar
Wash tubs
Sieve buckets
Plastic picking trays
Digital camera
Forceps
Meter (YSI Professional Plus)
Waders, rain gear
Vials, and containers for vials
Alcohol
Labels and collection cards, pencils
Habitat Assessment Forms
GPS Unit
First Aid Kit
Insect Repellant

B. Laboratory Equipment and Supplies

Dissecting microscopes
Compound microscopes
Alcohol
Formalin
Polyvinyl lactophenol (CMC Mounting Media) or Hoyer's Solution
Vials
Forceps
Cover slips
Microscope slides
Petri dishes
Squeeze bottles
Dissecting needles
Slide labels
Slide holders
Benthic Macroinvertebrate lab sheets

Appendix 3. Benthos Collection Card

BENTHOS COLLECTION CARD

DATE _____ **COLLECT TIME** _____ **COLLECTORS** _____ **CARD#** _____

WATERBODY _____

STAT. LOC. _____ **RIVER BASIN** _____ **COUNTY** _____

Substrate:		**River:**		**Field Parameters:**	
Boulder (10")	____ %	Mean depth	_____	Bank Erosion	N ____ Mod ____ Sev ____
Cobble (2 1/2-10")	____ %	Maxim. depth	_____	Canopy	% ____ Type _____
Gravel (1/12-2 1/2")	____ %	Width	_____	Aufwuchs	N ____ Mod ____ Abund. ____
Sand (1/12")	____ %	Current	_____	Podostemum	N ____ Mod ____ Abund. ____
Silt, fine Partic.	____ %	Recent Rain ?	_____	Tribs Present?	_____
Other	____ %	**Photos**	**(#)** _____		

Instream Habitat: (0,+,++)				**Samples: (#)**		**Water Chemistry:**	
Pools	_____	Backwaters	_____	Kicks	_____	Temperature (°C)	_____
Riffles	_____	Detritus	_____	Sweeps	_____	Dissolved Oxygen (mg/L)	_____
Snags	_____	Aquatic Weeds	_____	Leaf Packs	_____	Conductivity (µmhos/cm)	_____
Undercut Banks	_____	Other	_____	Rock-Log	_____	pH	_____
Root Mats	_____			Sand	_____		
				Visuals	_____		
				Other	_____		

Field Observations: _____

Appendix 4. Benthic Macroinvertebrate Lab Sheet

Benthic Macroinvertebrate Lab Sheet

Water Body		***Road/County***	
Sample Type		***CC Number***	
Date Collected		***Analyst***	
ID Start		***Collectors***	
ID Finish		***Date in database***	

Ephemeroptera	**N**	**A**	**Notes**
Plecoptera			
Trichoptera			

Diptera	**N**	**A**	**Notes**
Chironomidae			
Coleoptera			

Odonata	**N**	**A**	**Notes**
Oligochaeta			
Megaloptera			
Crustacea			
Mollusca			
Other			

Total Taxa	**0**
Total EPT	**0**
EPT N	
Biotic Index	
EPT BI	
Bioclass	

Notes:

SM—slide-mounted; NM—not mounted; EI—early instar; L—larva(e); INDET—indeterminate, doesn't fit key or descriptions for proper taxonomic effort; COND—condition; AGG—aggregated; #R—number ret. for personal ref. coll.

11/13 Revision 8

Habitat Assessment Field Data Sheet
Mountain/ Piedmont Streams

Biological Assessment Branch, DWR **TOTAL SCORE_____**

Directions for use: The observer is to survey a **minimum of 100 meters with 200 meters preferred** of stream, preferably in an **upstream** direction starting above the bridge pool and the road right-of-way. The segment which is assessed should represent average stream conditions. To perform a proper habitat evaluation the observer needs to get into the stream. To complete the form, select the description which best fits the observed habitats and then circle the score. If the observed habitat falls in between two descriptions, select an intermediate score. A final habitat score is determined by adding the results from the different metrics.

Stream_____Location/road: _____(Road Name_____)County_____

Date_____**CC#**_____Basin_____Subbasin_____

Observer(s)_____ Type of Study: ☐ Fish ☐Benthos ☐ Basinwide ☐Special Study (Describe) _____

Latitude _____Longitude _____Ecoregion: ☐ MT ☐ P ☐ Slate Belt ☐ Triassic Basin

Water Quality: Temperature_____^{0}C DO _____mg/l Conductivity (corr.) _____µS/cm pH _____

Physical Characterization: Visible land use refers to immediate area that you can see from sampling location - include what you estimate driving thru the watershed in watershed land use.

Visible Land Use: _____%Forest _____%Residential _____%Active Pasture _____% Active Crops _____%Fallow Fields _____% Commercial _____%Industrial _____%Other - Describe:_____

Watershed land use : ☐Forest ☐Agriculture ☐Urban ☐ Animal operations upstream

Width: (meters) Stream_____ Channel (at top of bank)_____ **Stream Depth**: (m) Avg_____Max ____
☐ Width variable ☐ Large river >25m wide
Bank Height (from deepest part of riffle to top of bank-first flat surface you stand on): (m)_____

Bank Angle: _____ º or ☐ NA (Vertical is 90º, horizontal is 0º. Angles > 90º indicate slope is towards mid-channel, < 90º indicate slope is away from channel. NA if bank is too low for bank angle to matter.)
☐ Channelized Ditch
☐Deeply incised-steep, straight banks ☐Both banks undercut at bend ☐Channel filled in with sediment
☐ Recent overbank deposits ☐Bar development ☐Buried structures ☐Exposed bedrock
☐ Excessive periphyton growth ☐ Heavy filamentous algae growth ☐Green tinge ☐ Sewage smell
Manmade Stabilization: ☐N ☐Y: ☐Rip-rap, cement, gabions ☐ Sediment/grade-control structure ☐Berm/levee
Flow conditions : ☐High ☐Normal ☐Low
Turbidity: ☐Clear ☐ Slightly Turbid ☐Turbid ☐Tannic ☐Milky ☐Colored (from dyes)
Good potential for Wetlands Restoration Project?? ☐ YES ☐NO Details_____
_____

Channel Flow Status

Useful especially under abnormal or low flow conditions.

A. Water reaches base of both lower banks, minimal channel substrate exposed ..... ☐
B. Water fills >75% of available channel, or <25% of channel substrate is exposed..... ☐
C. Water fills 25-75% of available channel, many logs/snags exposed..... ☐
D. Root mats out of water..... ☐
E. Very little water in channel, mostly present as standing pools..... ☐

Weather Conditions:_____**Photos:** ☐N ☐Y ☐ Digital ☐35mm

Remarks:_____
_____
_____
_____

Appendix 5. Habitat Assessment Field Data Sheet - Mountain/ Piedmont Streams

I. Channel Modification Score

A. channel natural, frequent bends..... 5
B. channel natural, infrequent bends (channelization could be old)..... 4
C. some channelization present..... 3
D. more extensive channelization, >40% of stream disrupted..... 2
E. no bends, completely channelized or rip rapped or gabioned, etc..... 0

☐ Evidence of dredging ☐Evidence of desnagging=no large woody debris in stream ☐Banks of uniform shape/height
Remarks_____ Subtotal____

II. Instream Habitat: Consider the percentage of the reach that is favorable for benthos colonization or fish cover. If >70% of the reach is rocks, 1 type is present, circle the score of 17. Definition: leafpacks consist of older leaves that are packed together and have begun to decay (not piles of leaves in pool areas). Mark as **R**are, **C**ommon, or **A**bundant.

____Rocks ____Macrophytes ____Sticks and leafpacks ____Snags and logs ____Undercut banks or root mats

AMOUNT OF REACH FAVORABLE FOR COLONIZATION OR COVER

	>70% Score	40-70% Score	20-40% Score	<20% Score
4 or 5 types present.....	20	16	12	8
3 types present.....	19	15	11	7
2 types present.....	18	14	10	6
1 type present.....	17	13	9	5
No types present.....	0			

☐ No woody vegetation in riparian zone Remarks_____ Subtotal_____

III. Bottom Substrate (silt, sand, detritus, gravel, cobble, boulder) Look at entire reach for substrate scoring, but only look at riffle for embeddedness, and use rocks from all parts of riffle-look for "mud line" or difficulty extracting rocks.

A. substrate with good mix of gravel, cobble and boulders **Score**

1. embeddedness <20% (very little sand, usually only behind large boulders)..... 15
2. embeddedness 20-40%..... 12
3. embeddedness 40-80%..... 8
4. embeddedness >80%..... 3

B. substrate gravel and cobble

1. embeddedness <20%..... 14
2. embeddedness 20-40%..... 11
3. embeddedness 40-80% 6
4. embeddedness >80%..... 2

C. substrate mostly gravel

1. embeddedness <50%..... 8
2. embeddedness >50%..... 4

D. substrate homogeneous

1. substrate nearly all bedrock..... 3
2. substrate nearly all sand 3
3. substrate nearly all detritus..... 2
4. substrate nearly all silt/ clay..... 1

Remarks_____Subtotal_____

IV. Pool Variety Pools are areas of deeper than average maximum depths with little or no surface turbulence. Water velocities associated with pools are always slow. Pools may take the form of "pocket water", small pools behind boulders or obstructions, in large high gradient streams, or side eddies.

A. Pools present Score

1. Pools Frequent (>30% of 200m area surveyed)
   a. variety of pool sizes..... 10
   b. pools about the same size (indicates pools filling in)..... 8
2. Pools Infrequent (<30% of the 200m area surveyed)
   a. variety of pool sizes..... 6
   b. pools about the same size..... 4

B. Pools absent..... 0

Subtotal_____

☐ Pool bottom boulder-cobble=hard ☐ Bottom sandy-sink as you walk ☐ Silt bottom ☐ Some pools over wader depth
Remarks_____

Page Total_____

Appendix 5. Habitat Assessment Field Data Sheet - Mountain/ Piedmont Streams

V. Riffle Habitats

Definition: Riffle is area of reaeration-can be debris dam, or narrow channel area.

	Riffles **Frequent** Score	Riffles **Infrequent** Score
A. well defined riffle and run, riffle as wide as stream and extends 2X width of stream....	16	12
B. riffle as wide as stream but riffle length is not 2X stream width	14	7
C. riffle not as wide as stream and riffle length is not 2X stream width	10	3
D. riffles absent.....	0	

Channel Slope: ☐Typical for area ☐Steep=fast flow ☐Low=like a coastal stream Subtotal_____

VI. Bank Stability and Vegetation

A. Erosion

1. No, or very little, erosion present 7
2. Erosion mostly at outside of meanders 6
3. Less than 50% of banks eroding 3
4. Massive erosion ..... 0 Erosion Score_____

B. Bank Vegetation

1. Mostly mature trees (>12" DBH) present 7
2. Mostly small trees (<12" DBH) present, large trees rare 5
3. No trees on bank, can have some shrubs and grasses 3
4. Mostly grasses or mosses on bank 2
5. Little or no bank vegetation, bare soil everywhere ..... 0 Vegetation Score_____

Remarks_____ Subtotal_____

VII. Light Penetration Canopy is defined as tree or vegetative cover directly above the stream's surface. Canopy would block out sunlight when the sun is directly overhead. Note shading from mountains, but not use to score this metric.

	Score
A. Stream with **good** canopy with some breaks for light penetration	10
B. Stream with **full canopy** - breaks for light penetration absent.....	8
C. Stream with **partial** canopy - sunlight and shading are essentially equal.....	7
D. Stream with **minimal** canopy - full sun in all but a few areas.....	2
E**. No canopy** and no shading.....	0

Remarks_____ Subtotal_____

VIII. Riparian Vegetative Zone Width

Definition: Riparian zone for this form is area of natural vegetation adjacent to stream (can go beyond floodplain). Definition: A break in the riparian zone is any place on the stream banks which allows sediment or pollutants to directly enter the stream, such as paths down to stream, storm drains, uprooted trees, otter slides, etc.

FACE UPSTREAM

Dominant vegetation: ☐ Trees ☐ Shrubs ☐ Grasses ☐ Weeds/old field ☐Exotics (kudzu, etc)

	Lft. Bank Score	Rt. Bank Score
A. Riparian zone **intact** (no breaks)		
1. width > 18 meters.....	5	5
2. width 12-18 meters.....	4	4
3. width 6-12 meters.....	3	3
4. width < 6 meters.....	2	2
B. Riparian zone **not intact** (breaks)		
1. breaks rare		
a. width > 18 meters.....	4	4
b. width 12-18 meters.....	3	3
c. width 6-12 meters.....	2	2
d. width < 6 meters.....	1	1
2. breaks common		
a. width > 18 meters.....	3	3
b. width 12-18 meters.....	2	2
c. width 6-12 meters.....	1	1
d. width < 6 meters.....	0	0

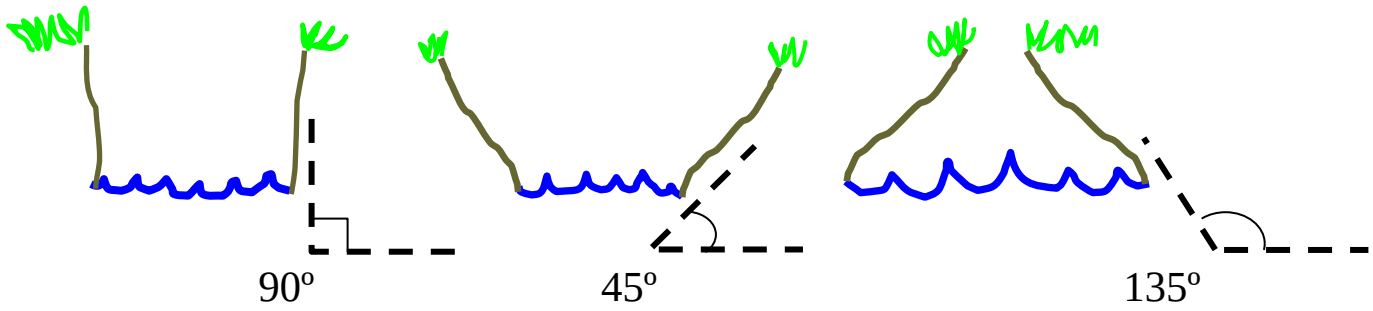
Remarks_____ Subtotal_____

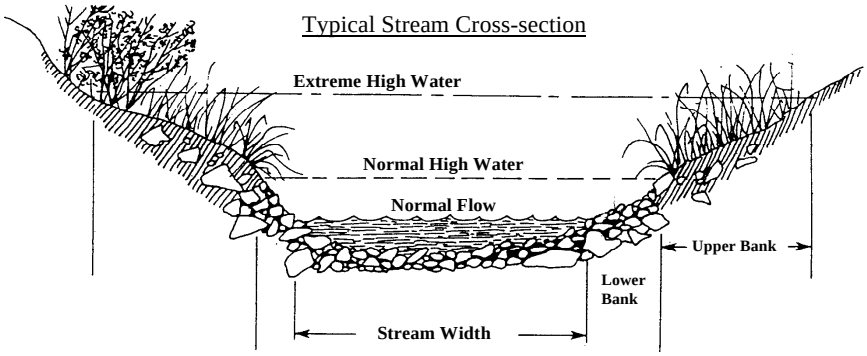
Page Total_____

☐ Disclaimer-form filled out, but score doesn't match subjective opinion-atypical stream. **TOTAL SCORE_____**

Supplement for Habitat Assessment Field Data Sheet

Diagram to determine bank angle:





This side is 45º bank angle.

Site Sketch:

Other comments:_____

_____

_____

_____

_____

11/13 Revision 9

Habitat Assessment Field Data Sheet
Coastal Plain Streams

TOTAL SCORE_____

Biological Assessment Branch, DWR

Directions for use: The observer is to survey a **minimum of 100 meters with 200 meters preferred** of stream, preferably in an **upstream** direction starting above the bridge pool and the road right-of-way. The segment which is assessed should represent average stream conditions. To perform a proper habitat evaluation the observer needs to get into the stream. To complete the form, select the description which best fits the observed habitats and then circle the score. If the observed habitat falls in between two descriptions, select an intermediate score. A final habitat score is determined by adding the results from the different metrics.

Stream_____Location/road: _____(Road Name_____)County_____

Date_____**CC#**_____Basin_____Subbasin_____

Observer(s)_____ Type of Study: ☐ Fish ☐Benthos ☐ Basinwide ☐Special Study (Describe) _____

Latitude _____Longitude _____Ecoregion: ☐ CA ☐ SWP ☐ Sandhills ☐ CB

Water Quality: Temperature_____^{0}C DO _____mg/l Conductivity (corr.) _____µS/cm pH _____

Physical Characterization: Visible land use refers to immediate area that you can see from sampling location. Check off what you observe driving thru the watershed in watershed land use.

Visible Land Use: _____%Forest _____%Residential _____%Active Pasture _____% Active Crops
_____%Fallow Fields _____% Commercial _____%Industrial _____%Other - Describe:_____

Watershed land use ☐ Forest ☐ Agriculture ☐Urban ☐ Animal operations upstream

Width: (meters) Stream_____ Channel (at top of bank)_____ **Stream Depth**: (m) Avg_____Max ____
☐ **Width variable** ☐**Braided channel** ☐**Large river >25m wide**
Bank Height (from deepest part of channel to top of bank): (m)_____

Flow conditions : ☐High ☐Normal ☐Low
Channel Flow Status

Useful especially under abnormal or low flow conditions.

A. Water reaches base of both banks, minimal channel substrate exposed ..... ☐
B. Water fills >75% of available channel, or <25% of channel substrate is exposed..... ☐
C. Water fills 25-75% of available channel, many logs/snags exposed..... ☐
D. Root mats out of water..... ☐
E. Very little water in channel, mostly present as standing pools..... ☐

Turbidity: ☐Clear ☐ Slightly Turbid ☐Turbid ☐Tannic ☐Milky ☐Colored (from dyes) ☐Green tinge
Good potential for Wetlands Restoration Project?? ☐ YES ☐ NO Details_____
_____

☐Channelized ditch
☐Deeply incised-steep, straight banks ☐Both banks undercut at bend ☐Channel filled in with sediment
☐Recent overbank deposits ☐Bar development ☐Sewage smell
☐Excessive periphyton growth ☐Heavy filamentous algae growth

Manmade Stabilization: ☐N ☐Y: ☐Rip-rap, cement, gabions ☐ Sediment/grade-control structure ☐Berm/levee
Weather Conditions:_____**Photos:** ☐N ☐Y ☐Digital ☐35mm

Remarks:_____
_____
_____

TYPICAL STREAM CROSS SECTION DIAGRAM ON BACK

Appendix 6. Habitat Assessment Field Data Sheet – Coastal Plain Streams

I. Channel Modification

	Score
A. Natural channel-minimal dredging.....	15
B. Some channelization near bridge, or historic (>20 year old), and/or bends beginning to reappear..	10
C. Extensive channelization, straight as far as can see, channelized ditch.....	5
D. Banks shored with hard structure, >80% of reach disrupted, instream habitat gone.....	0

Remarks_____ Subtotal____

II. Instream Habitat: Consider the percentage of the reach that is favorable for benthos colonization or fish cover. If >50% of the reach is snags, and 1 type is present, circle the score of 16. Definition: leafpacks consist of older leaves that are packed together and have begun to decay (not piles of leaves in pool areas). Mark as **R**are, **C**ommon, or **A**bundant.

____Sticks ____Snags/logs ____Undercut banks or root mats ____Macrophytes ____Leafpacks

AMOUNT OF REACH FAVORABLE FOR COLONIZATION OR COVER

	>50% Score	30-50% Score	10-30% Score	<10% Score
4 or 5 types present.....	20	15	10	5
3 types present.....	18	13	8	4
2 types present.....	17	12	7	3
1 type present.....	16	11	6	2
No substrate for benthos colonization and no fish cover.....				0

☐ No woody vegetation in riparian zone Remarks_____ Subtotal_____

III. Bottom Substrate (silt, clay, sand, detritus, gravel) look at entire reach for substrate scoring.

	Score
A. Substrate types mixed	
1. gravel dominant.....	15
2. sand dominant.....	13
3. detritus dominant.....	7
4. silt/clay/muck dominant.....	4
B. Substrate homogeneous	
1. nearly all gravel.....	12
2. nearly all sand	7
3. nearly all detritus.....	4
4. nearly all silt/clay/muck.....	1

Remarks_____Subtotal_____

IV. Pool Variety Pools are areas of deeper than average maximum depths with little or no surface turbulence. Water velocities associated with pools are always slow.

	Score
A. Pools present	
1. Pools Frequent (>30% of 100m length surveyed)	
a. variety of pool sizes.....	10
b. pools about the same size (indicates pools filling in).....	8
2. Pools Infrequent (<30% of the 100m length surveyed)	
a. variety of pool sizes.....	6
b. pools about the same size.....	4
B. Pools absent	
1. Deep water/run habitat present.....	4
2. Deep water/run habitat absent.....	0
	Subtotal_____

Remarks_____ Page Total_____

V. Bank Stability and Vegetation

A. Erosion

1. No, or very little, erosion present 10
2. Erosion mostly at outside of meanders 6
3. Less than 50% of banks eroding 3
4. Massive erosion ..... 0 Erosion Score_____

B. Bank Vegetation

1. Mostly mature trees (>12" DBH) present 10
2. Mostly small trees (<12" DBH) present, large trees rare 7
3. No trees on bank, can have some shrubs and grasses 4
4. Mostly grasses or mosses on bank 3
5. Little or no bank vegetation, bare soil everywhere ..... 0 Vegetation Score_____

Remarks_____ Subtotal_____

VI. Light Penetration (Canopy is defined as tree or vegetative cover directly above the stream's surface. Canopy would block out sunlight when the sun is directly overhead).

	Score
A. Stream with **good** canopy with some breaks for light penetration	10
B. Stream with **full canopy** - breaks for light penetration absent.....	8
C. Stream with **partial** canopy - sunlight and shading are essentially equal.....	7
D. Stream with **minimal** canopy - full sun in all but a few areas.....	2
E. **No canopy** and no shading.....	0

Subtotal_____

Remarks_____

VII. Riparian Vegetative Zone Width

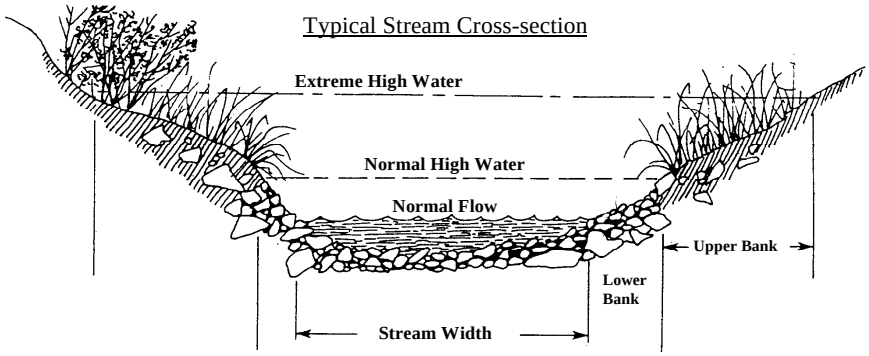
Definition: A break in the riparian zone is any area which allows sediment to enter the stream. Breaks refer to the near-stream portion of the riparian zone (banks); places where pollutants can directly enter the stream.

	Lft. Bank Score	Rt. Bank Score
A. Riparian zone **intact** (no breaks)		
1. zone width > 18 meters.....	5	5
2. zone width 12-18 meters.....	4	4
3. zone width 6-12 meters.....	3	3
4. zone width < 6 meters.....	2	2
B. Riparian zone **not intact** (breaks)		
1. breaks rare		
a. zone width > 18 meters.....	4	4
b. zone width 12-18 meters.....	3	3
c. zone width 6-12 meters.....	2	2
d. zone width < 6 meters.....	1	1
2. breaks common		
a. zone width > 18 meters.....	3	3
b. zone width 12-18 meters.....	2	2
c. zone width 6-12 meters.....	1	1
d. zone width < 6 meters.....	0	0

Remarks_____ Subtotal_____

Page Total_____

TOTAL SCORE _____



This side is 45º bank angle.