Standard Operating Procedures for the Collection and Analysis of Benthic Macroinvertebrates

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NORTH CAROLINA DEPARTMENT OF ENVIRONMENTAL QUALITY

Division of Water Resources
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REVISION LOG

Date Edited	Editor	Version Edited	Section Edited	Changes/updates
May 2015	Steven Kroeger & Hannah Headrick	Ver. 4.0	Entire Document	Minor edits, and document formatting
15 May 2015	Michael Walters	Ver. 4.0	PRE-FIELD PREPARATION AND CONSIDERATIONS: Health and Safety	Changed "Environmental Sciences Section" to "Water Sciences Section." Clarified that the safety committee applicable to BAB is restricted to the 4401 Reedy Creek Rd building within WSS. Added hydration and sunblock use as needs to prevent illness and injury during field outings. Added link to department instructions for reporting injuries.
15 May 2015	Michael Walters	Ver. 4.0	SAMPLING PROCEDURE: Sample Collection Methods	Under Standard Qualitative (now Full Scale) Method, added a line indicating that unionid mussels are photographed and returned to the stream. Removed sentence under Swamp Method stating that each sweep should be emptied into a tub.
15 May 2015	Michael Walters, Eric Fleek	Ver. 4.0	PRE-FIELD PREPARATION AND CONSIDERATIONS: Equipment and Supplies	Changed the mesh size for the triangle frame sweep net (subsection Sampling Equipment) from 1000 micron to 800-900 micron after consulting the Wildlife Supply Company (our supplier for the nets) website for specifications. Also within the subsection, put all equipment photos on a single page.
15 May 2015	Michael Walters, Eric Fleek	Ver. 4.0	LABORATORY TECHNIQUES AND DATA INTERPRETATION: Midge Deformity Analysis	Significant rewrites for clarification. Citations for published references added.
15 May 2015	Michael Walters	Ver. 4.0	QUALITY ASSURANCE	Added text to clarify the need to calibrate and check meters each day when performing fieldwork. Replaced last paragraph regarding the scoring of taxonomic QAs with updated version.
15 May 2015	Michael Walters	Ver. 4.0	REFERENCES	Added several references regarding midge deformities in the presence of environmental stressors.
15 May 2015	Michael Walters	Ver. 4.0	Appendices	Reworked and updated.

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October 2015	Jen Schmitz	Ver. 5.0	Entire document	Reorganization, document template and styles, rewriting major sections, added sections. Updated taxonomic lists and tolerance values.
November 2015	Michael Walters	Ver. 5.0	Entire document	Minor formatting and grammatical changes.
February 2016	Jen Schmitz	Ver. 5.0	Entire document	Final formatting and resolution.

FIGURES

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APPENDICES

ACRONYMS AND ABBREVIATIONS

BAB	Biological Assessment Branch
BI	Biotic Index
CC	collection card
DA	drainage area
DWR	Division of Water Resources
EPT	Ephemeroptera, Plecoptera, Trichoptera
HQSMS	high quality small mountain stream
HQW	high quality waters
ISB	Intensive Survey Branch
GPS	Geographic positioning system
NCDWQ	North Carolina Division of Water Quality
NTU	Nephelometric turbidity unit
ORW	outstanding resource waters
PVC	polyvinyl chloride
QA	quality assurance
QC	quality control
RO	regional office
SiteID	site identification
SOP	standard operating procedures
SR	secondary road
USEPA	United States Environmental Protection Agency
WRC	Wildlife Resource Commission

1.0 INTRODUCTION AND PURPOSE

The purpose of this manual is to provide details on routine standard operating procedures (SOPs) of the Biological Assessment Branch (BAB) of the North Carolina Division of Water Resources (DWR) for the collection and analysis of freshwater benthic macroinvertebrate data that may result in bioclassification (ratings). 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 developed from the experience of personnel within the branch. These methods have been shown to provide repeatable and useable data for water quality evaluations.

Benthic macroinvertebrates, especially aquatic insects, are associated with the substrates of streams, rivers, and lakes. The BAB uses aquatic macroinvertebrate biological surveys as one type of indicator of biological integrity in streams and rivers. Physical and/or chemical water quality surveys do not integrate fluctuations in water quality between sampling events. Therefore, short-term critical events may often be missed when relying upon physicochemical data alone. 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. Other species will have multiple generations per year. Therefore the biota, especially benthic macroinvertebrates, reflect both long and short term environmental conditions.

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 the presence of biological degradation, they are less useful for identifying the specific source(s) of degradation. Linking biological effects with their causes is particularly complex when multiple stressors impact a waterbody (USEPA 2000).

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. Additional studies have shown the biological impacts of sedimentation and toxic stress (Burton 1991, Waters 1995, Bode and Simpson 1982, Clements 1994).

Allowances during assessment must be made for stream size, geographic location, and seasonality. Also, flow conditions are related to the relative impacts due to point and nonpoint sources as 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.

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 that 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).

This manual is reviewed regularly and revised as necessary. The prior approved version of this manual was dated December 2013. All current employees and new employees within BAB will be provided with this manual to serve as a guideline for activities, methods, and procedures. SOPs and quality control (QC) procedures 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 an appropriate report or memo.

2.0 PRE-FIELD PREPARATION AND CONSIDERATIONS

In order to maximize collection efficiency, a trip itinerary is developed before sampling begins. Regional Office personnel are advised before any sampling trip as to where and when work will be done in their region. Once a sampling team (required number of staff will vary based on method used) has been assembled and field date has been selected, the benthos database and applicable BAB documents should be reviewed to best locate sampleable areas. The trip leader should also use the Internet to check stream stage height from the closest USGS gage station before traveling to the site.

The following sections outline protocols and procedures that should be considered when planning and implementing any field trip.

2.1 Health and Safety

Benthic macroinvertebrates are collected from rivers and streams throughout North Carolina, and at times and places where medical facilities may not be readily available. The Water Sciences Section – Biology Lab has a safety committee that is responsible for maintenance and development of current safety procedures and checklists to which all personnel are required to adhere. All employees are instructed to follow these safety precautions when using equipment and hazardous materials.

The following items are discussed with staff before fieldwork is conducted to minimize the likelihood of illness or injury:

- **Insect Repellent:** Staff will be provided with and should use insect repellent. 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.
- **Sun Protection:** Staff will be provided with and should use sunscreen to limit the deleterious effects of exposure to ultraviolet radiation.
- **Heat Stress:** Staff will be provided with and should use water and drinks designed to compensate for salt and water lost through strenuous activity in hot weather.
- **Visibility:** Staff will be provided with and should use reflective safety vests which should be worn whenever working on bridges or near traffic.
- **General Safety:** Turbidity (NTU) > 20 NTU denotes cloudy waters that, in addition to being unsuitable for benthic macroinvertebrate or water quality sampling, may be unsafe for in-water work. Caution should be used in this condition, particularly at medium to large river sites.

Sampling conditions are the primary safety factor to be considered when conducting field work. If any field conditions, such as high flows or thunderstorms, raise the question as to whether a sample can be collected safely, then a decision considering the safety of the personnel should be assessed. 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.

All vehicles are provided with first aid kits that are 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. Detailed instructions for the reporting of injuries are available through the department intranet and should be consulted (http://portal.ncdenr.org/group/srm/incidentreporting). The safety committee maintains a written record of accidents.

2.2 Personnel Qualifications

An experienced benthic biologist trained and skilled in field benthic sampling methods and organism identification must be present for all sample collections. Personnel must know insect morphology; be capable of reading dichotomous keys; and have knowledge of the benthic macroinvertebrate taxa found throughout North Carolina and in various stream habitats. 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, sample picking (look through trays again), and visuals.

2.3 Equipment and Supplies

It is vital to have the proper equipment and supplies on hand for successful sample collection and analysis, both in the field and in the laboratory. The items below are representative of typical equipment and supply needs during benthic macroinvertebrate sample collection, assessment, and analysis, which shall be obtained, inventoried, and maintained prior to sampling.

Table 1. Collection Equipment

Field Supplies	
Kick nets with 1000 micron mesh and weighted leading edge	Triangle frame sweep nets with 800-900 micron Nitex™ mesh
Sand sampler – rectangular frame net with 300 micron Nitex™ mesh	Fine-mesh sampler - 300 micron Nitex™ mesh placed between four inch PVC pipe fittings, and tall round plastic container into which the PVC device will fit
Sieve buckets with 600 micron mesh (US Standard No. 30)	Petite Ponar (only for boat sampling)
Wash tubs and picking trays	Forceps
6-dram glass vials with polyseal screw caps	Plastic containers with tightly sealing lids large enough to hold several 6-dram vials (2/crewmember)
Ethyl alcohol for sample preservation	Labels and collection cards, pencils
Digital camera	GPS unit
Water quality meter (YSI Professional Plus)	
Supporting Supplies	
Waders and rain gear	Insect Repellant
Sun screen	First Aid Kit
Shoulder-length rubber gauntlets	
Laboratory Supplies	
Dissecting microscopes	Squeeze bottles
Compound microscopes	Dissecting needles
Microscope slides	Slide labels and holders
Forceps	Benthic macroinvertebrate lab sheets
Petri dishes	Cover slips
Glass vials	Polyvinyl lactophenol (CMC Mounting Media) or Hoyer's Solution

Kick Net

Sweep Net

Sand Sampler

Fine-Mesh Sampler and Container

Fine-Mesh Sampler disassembled

Sieve Bucket, Tub, Sample Container

Picking Tray, Vial, Forceps

Figure 1. Photographs of representative sampling equipment

2.3.1 Calibration and Standardization

All meters and other equipment must be calibrated in the lab or other controlled environment (e.g., hotel room) and a lab calibration form completed before being taken into the field each day. Lab calibration forms are given to the BAB Supervisor. Meters must be checked after sampling each day using laboratory standards and buffers for specific conductance and pH. 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. The electronic form is available on a shared DWR network drive[1]. Detailed information on calibration requirements can be found in the standard operating procedures for physical and chemical monitoring used by the Intensive Survey Branch[2].

[1] T:\QA QC\Meters & Calibration

[2] http://portal.ncdenr.org/c/document_library/get_file?uuid=516f1b7b-fbb6-419f-83c8-0c981b2e1f78&groupId=38364

3.0 SAMPLING PROCEDURE

3.1 Overview

The following are primary elements to be considered when performing sampling, which are also discussed further throughout the document:

- **Documentation.** Proper documentation of site layout and conditions is required. Photographs of the site must be taken, and a site sketch should be made that shows any unique habitats for those basin assessment locations that do not have site sketches. This sketch should include enough detail that subsequent field crews can return to the same sampling location.
- **High Flow Conditions**. Most of the sampling methodologies described in this manual require that freshwater streams or rivers be wadeable for safe and effective 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 bioclassification. If high water makes sampling conditions unsafe, it is better to return to the site during a more appropriate flow regime.
- **Low Flow.** Drought conditions can play a major role in altering the composition of the benthic fauna. Every effort should be made in locations 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 recent flow conditions have been extremely high or low.
- **Physicochemical measurements**. Water temperature, pH, conductivity, and dissolved oxygen measurements are obtained and recorded.
- **Sensitive Species.** The WRC has 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.
- **Methodology**. All samples are collected as described in Sample Collection Methods (Section 3.3).
- **Chain of Custody.** Sample containers are labeled before leaving the site with waterbody name, station location, collection card number, initials of collectors, and date of collection. Custody of the sample containers is maintained by DWR staff at all times.

3.2 Pre-sampling Site Assessment

3.2.1 Conditions and Flow

One of the most important steps to complete before sampling a site is to survey it from the bridge to evaluate the upstream and downstream segments for adequate available habitat (i.e. the reach is mostly wadeable, with one or two riffles present with movable substrate, and discernible flow over much of the reach) before selecting a reach to sample. Simultaneous examination of the area for the safest route to access the chosen sample reach should be performed. Consulting the individual site descriptions from prior sampling events is an invaluable tool to further aid in this process.

Physical stream form, habitat availability, and habitat quality can vary widely between ecoregions. 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 (where “bridge-pool” is defined as the pool within the stream that is very often present at the road crossing and may extend several tens of meters in the upstream and downstream directions) is often required to find suitable flow and habitat. The lack of flow at the bridge-pool may not be representative of flow conditions within the entire sampling reach. Hiking upstream or

downstream is often necessary to find flow in these systems. Consulting the notes for prior sampling events at a site, either in the database or on the BAB network drive[3], is invaluable for locating areas suitable for sampling, as well as manageable routes of access to the stream.

3.2.2 Habitat Assessment

A habitat assessment should be completed for all collections using the directions given on the habitat assessment form. In most areas, it is obvious whether the Mountain/Piedmont habitat form (Appendix A) or the Coastal Plain habitat form (Appendix B) should be used. In some transitional areas, however, a field decision must be made as to which form to use. If the stream is naturally rocky with a riffle-pool sequence then the Mountain/Piedmont habitat form should be used, even if the Level IV ecoregion (Griffith *et al.* 2002) 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.

A few particular points with regard to the habitat form:

- For the station location, a Secondary Road (SR) number is often used. Note that SR numbers will change at county lines and that the appropriate county must be recorded on the habitat form (as well as the collection card). The NC Department of Transportation Secondary Road database can be consulted to help determine correct SR numbers from road names: https://apps.ncdot.gov/srlookup/.
- Latitude and longitude should be determined using a handheld GPS unit with the geodetic reference set to NAD83 (North American Datum 1983).
- Ecoregions on the Coastal Plain habitat may not be intuitive. Region definitions for that form are:
    - CA: ("Coastal A"). Indicates a stream site with continuous flow throughout the year east of the fall line exclusive of the Sand Hills, and for which Full Scale, EPT, or Qual-4 sampling methods are used.
    - SWP: Indicates a stream site where there is normally not continuous flow throughout the year and for which the swamp collection method is used. Consult the section Swamp Stream Criteria (page 26) for more details on when and where such sites can receive a bioclassification.
    - Sand Hills: Same as for CA, except the site is located within the Sand Hills ecoregion.
    - CB: ("Coastal B"). Indicates an unwadeable large-river site for which the Boat collection method is used ("B" refers to "boat").

In addition to above assessment, an evaluation of benthic quality should be performed. Therefore, the benthos collection card (Appendix C) must be filled out. Field observations should include:

- ***Immediate watershed***. Record type of land use, extent of disturbed land, any floodplain deposition of sediment, any evidence of stream widening and/or infill, 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/or sediment on vegetation above water level, any livestock with access to stream, any point sources, and any unique habitats.
- ***Substrate***. Two collectors make independent estimates of substrate percentages and the independent and average values recorded on the collection card. Also note embedded substrate (interstitial spaces filled 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

[3] (R:\BASINWIDE SITE NOTES & LOCATIONS)

of unstable coarse sand or movement of bedload material, and amount of substrate covered with *aufwuchs* or silt.

- ***Width***. 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. 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 meter. Any unusual characteristics, such as a braided channel in coastal areas, should also 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 bryozoans, sponges, and mussel shells. Note any organisms that are very abundant. Note if diversity is limited to banks and snags above the effects of sediment scour.

All samples are transported to the Water Sciences Section – Biology Laboratory 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 number depending on the collection method used. The sampling techniques outlined here usually take 4-6 person hours (i.e., 1.5 to 2 hours per site with three collectors) for the Full Scale 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 or flow conditions.

3.2.3 Physicochemical measurements

A calibrated YSI Professional Plus meter is used to measure the following: temperature, dissolved oxygen, specific conductance, and pH. The four values are recorded on both the habitat form and the collection card.

3.3 Sample Methods

In order to decide the most appropriate sample collection method, 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. For each method, invertebrates are separated ("picked") from the rest of the sample in the field using forceps and picking trays, and preserved in glass vials containing 95% ethyl alcohol. Organisms are picked roughly in proportion to their abundance, but no attempt is made to remove all specimens. 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.

To reduce processing times in the laboratory, it is wise to minimize the amount of extraneous detritus and organic material included in the sample. To further reduce both field collection and laboratory processing effort, it is important during the field pick to communicate effectively to avoid over-picking taxa that are abundant and easily identified in the field.

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 their 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.

Due to the difficulty with identification in the field, and the presence of threatened and endangered species in North Carolina, unionid mussels are photographed then returned to the stream.

Four different macroinvertebrate collection methods are used to collect benthic samples from wadeable streams: Full Scale Method; EPT Method; Qual 4 Method; and Swamp Method. Descriptions of each method are below.

3.3.1 Full Scale Method

The Full Scale method can be used to assign water quality ratings (bioclassifications) 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 303(d) list) for which the drainage area is over 3.0 square miles.

For the Full Scale method the following collections are made (which are described in further detail in Section 3.4):

- two riffle-kicks
- three sweeps
- one leaf-pack
- two rock- and log-washes
- one sand
- visual

3.3.2 EPT Method

The EPT method is an abbreviated version of the Full Scale method and 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.

Ephemeroptera Plecoptera Trichoptera

Figure 2. Photographs illustrating EPT taxa

The collection and analysis time for the EPT method has been decreased from the Full Scale method in two ways. First, collections focus solely on a subset of the benthic community composed of taxa in the taxonomic orders Ephemeroptera, Plecoptera, and Trichoptera (Figure 2). These orders usually include the most intolerant species among benthic invertebrates. 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 to four:

- one riffle-kick
- one sweep
- one leaf-pack
- visual

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. EPT samples are also inappropriate for sites that require a biotic index calculation (page 19).

3.3.3 Qual 4 Method

This method uses the same collection techniques as the EPT method, but all representative specimens of the entire macroinvertebrate community (i.e., not just EPT taxa) are picked from the collections. Using the Qual 4 method, four collections are made:

- one riffle-kick
- one sweep
- one leaf-pack
- visual

This method was designed to be used only in small streams, which are defined as those sites having a drainage area (DA) ≤ 3.0 square miles (NCDWQ 2009). Such streams will likely have few EPT taxa, especially under oligotrophic conditions.

3.3.4 Swamp Method

This method is used for "swamp streams," which are defined by BAB as those streams that are within the coastal plain ecoregion and normally do not have visible flow during a part of the year. This period of little or no flow usually occurs during summer months, but flowing water should be present in swamp streams during the winter months. Sampling during the winter high-flow period provides the best opportunity for detecting differences in communities from what is natural, therefore criteria were developed for February to early March and only data from benthic collections made during this period can be used when evaluating swamp streams. In addition, swamp streams with pH values of 4.0 or lower are not rated; even those below 4.5 are difficult to evaluate.

The swamp sampling method utilizes a variety of collection techniques to inventory the macroinvertebrate community at a site. A total of nine sweep collections are made 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. Three log/debris washes are also collected. Visual collections are the final technique used at each site.

The methods outlined here, among others, are utilized in various sampling scenarios as described in the following sections.

3.4 Wadeable Stream Collection Methods

The majority of BAB sampling is conducted in wadeable waters. One or more of the collection methods described in Section 3.3 will be employed in the stream using several different techniques:

3.4.1 Riffle-kick Collection

Equipment Required: kick net

Approximately two meters of substrate should be disturbed for a typical riffle-kick collection. 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. that are particularly adept at maintaining robust contact with substrates. In addition, if *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 collections are required, two separate riffle areas should be targeted. If at all possible, avoid taking two riffle-kicks from the same riffle. Ideally, one riffle-kick should be obtained in fast water and another in slower water; avoid taking the two riffle-kicks 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 collections includes substrate harboring this vegetation. For riffle-kick collections, 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-kick collections 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 collection.

Before initiating a riffle-kick, make certain the bottom of the net is in full contact with the bottom of the stream channel. 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 such instances, obtain rocks from outside of the riffle collection area and use them to weigh the bottom of the net down. While sampling, it is also a good idea to remove fresh ("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.

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 be dry during lower flow conditions. In such instances, 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.

After the collection has been obtained, it is advisable to fold the net over itself to keep organisms 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.

3.4.2 Sweep Collection

Equipment Required: 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) that 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 collection type to collect from the habitat starting downstream and working in the upstream direction. This practice will prevent sediment and turbidity from being introduced into uncollected habitat downstream. 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 collection 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.

3.4.3 Leaf-pack Collection

Equipment Required: sieve bucket

Unlike most of the previously discussed collection methods, leaf-packs are typically restricted to areas of strong flow. As a result, the investigator should not expend too much effort searching for leaf-packs 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 collected together with suitable material, it should be discarded during the elutriation process. Leaf packs 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* is the dominant leaf 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 sand hills, American Holly is often a large constituent of leaf pack material; while this is suitable substrate if conditioned, effort should also be made to target deciduous material when possible. Sticks are often collected with leaf material, but are not the primary target of this collection. Unless it is the only material present, grass clippings and other weedy debris should not be used as part of this collection type.

Obtaining a 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 leaf-packs in the deepest areas of the flow to ensure maximum exposure to water. Avoid taking your entire collection from just one area—it is always best to take from multiple packs (regardless of type) from various areas to ensure as representative a collection 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 two inches 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 two to three inches in depth. During the elutriation process, it is advisable to "rinse" the elutriated material by filling the bucket to within one inch of the top and rotating 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 collection 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, this elutriation process also serves to reduce the overall volume of material in the habitat which removes refugia for organisms and makes them more accessible for sampling.

3.4.4 Rock- and Log-wash Collection

Equipment Required: fine-mesh sampler, plastic tub, ethyl alcohol

The primary purpose of this sampling method is to target substrate sizes and flow regimes that were either missed or under-collected during the previous collection types. For example, large boulders might not be included in a cobble riffle 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 using the other collection methods. Although larger substrates are generally the favored type of habitat for this sample, smaller substrate sizes are also required for a representative collection.

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 wash-water representing a mix of material and water are poured through the fine-mesh sampler. 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 fine-mesh sampler with the tub. Manually scraping the receiving surface of the filter with a finger can also be an effective way to prevent clogging.

This collection 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 these situations, care should be exercised and only the most ideal 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 collection, simply place the fine-mesh sampler into a container with enough ethyl alcohol to inundate the collected material. This should be allowed to stand for at least 15 minutes before picking. This process will ensure the small chironomids will be motionless and will cause most to float to the surface and will make organisms easier to collect.

3.4.5 Sand Collection

Equipment Required: sand sampler, fine-mesh sampler, plastic tub, ethyl alcohol

This collection method specifically targets sand. In those 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 simply disturb the sand (or the rocks or logs where the sand is accumulated interstitially) and allow the disturbed material to collect in the net. When possible, avoid collecting areas of heavy organic deposition (leaf packs, sticks, *Podostemum*) since heavy collections of these materials will make the elutriation and filtering of the collection more problematic. Approximately a softball 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, swirl the material around several times, and quickly pour off the suspended sediment (and organisms contained within) through the chironomid fine-mesh sampler.

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

3.4.6 Visuals

Equipment Required: forceps, vial filled with 95% ethyl alcohol

Three investigators participate in this portion of the field collection, which is done after all other collections have been made and field-picked. If a two-member crew is performing the collection, then the time spent on this collection by each investigator should be increased as appropriate. Approximately 10-15 minutes should be allotted for visual collections. Often, in areas of very poor habitat or very poor diversity, 10 minutes will provide more than enough time to obtain a representative collection. In areas of high habitat heterogeneity or very high diversity, 15 minutes may not allow for enough time to obtain a good collection. The intent of this sample type is to specifically target microhabitats that were either not sampled from any of the aforementioned collection methods or were under-collected. Examples generally include very large substrates (e.g., logs and boulders) in areas of very high or very low flow. 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 often still 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, 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 that are 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 riffle-kick or wash collections, so effort must be made to target them during visuals. Similarly, many mayflies (e.g., *Epeorus*) that 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*.

It is strongly advised to pack at least three extra vials to be used solely for visual collections; this prevents loss in the event of a dropped vial. Visual collections are made at the end of the assessment in order to facilitate the identification of under-sampled habitats.

3.5 Boat Sample Method

While most samples are collected from wadeable streams, 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 collections replaces kick-net collections, but all other Full Scale 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 (or “Boat”) criteria are used to evaluate such sampling sites.

As with the Full Scale method, the Boat Sampling method aims at a total of 10 composited collections per site. Efficiency is maximized by leaving one or two investigators on shore to do the sweep collections and a portion of the epifaunal, visual, and leaf pack/debris collections. The remaining investigators make the petite ponar collections and a portion of the leaf pack/debris, epifaunal, and visual collections from logs in flow. When the shore area is very steep, some sweeps may be collected from the boat, although this can be less effective than wading.

- **Petite Ponar.** Three locations between midstream and the banks are collected using a petite ponar sampler, with three replicates at each location (a total of nine collections). Some portions of collections, such as those containing mostly organic material, may be processed without the use of a fine-mesh sampler; however, all other portions, such as sandy collections, should be elutriated and processed through a fine-mesh sampler. If possible, the three locations should be at a variety of depths, with at least one location in the two- to three-meter depth range. No petite ponar collections should be made from the depth normally sampled during shore work (i.e. less than two meters in depth). The petite ponar should be lowered slowly to avoid disturbance of surface sediments. The shallow collections are often good habitat for *Hexagenia* and *Phylocentropus*. Collection card notes should include the depths sampled and the general substrate composition at each location. Large clams (such as *Corbicula* and unionids) can be identified, recorded on the collection card, and returned to the waterbody.
- **Sweep**. Three sweeps will be collected from bank habitats at each site while collecting as much of the edge habitat as possible. If aquatic macrophytes are present, then these should be collected in one of the three sweeps. Other areas to be collected 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-pack and Debris** (one composited set of collections). 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 collected.
- **Epifaunal** (two composited collections). Macrophytes and well-colonized logs (both in the current and along the shore) should be washed and processed through the fine-mesh sampler. As usual, this is aimed at getting a good collection 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.
- **Visual** (one composited set of three collections). A fairly large proportion of the EPT fauna is often 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 a greater number of macroinvertebrate taxa. 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 visual collections must be conducted from the boat in most cases, and should be continued until several well-colonized logs have been found. Look 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 some very large logs into the boat. If the log can be lifted easily, it is probably too small. Colonization by Hydropsychidae is a good sign, but also look for Heptageniidae, Baetidae, Plecoptera (especially *Acroneuria* and *Neoperla*), and sand-cased Trichoptera.

3.6 Post-collection Procedures

3.6.1 Chain of Custody

All samples collected will be labeled clearly and placed in containers that will be kept in DWR custody at all times. The sample identification can consist of sample tags, labels, or indelible writing directly on the sample container. The sample information will include:

- unique field sample number (i.e. collection card number)
- date and time of sample collection
- names of field collectors

A field data sheet will serve as a chain of custody form and will be used to transfer custody of samples to the laboratory. This paperwork will remain with the samples at all times, and will remain in the custody of DWR staff until processed at the laboratory.

3.6.2 Other tasks

Before leaving a site, make absolutely certain that there are no organisms adhering to collection or processing surfaces. Organisms from one site should never be transferred to another site. To prevent this, rinse and examine all nets, buckets, trays, tubs, and fine-mesh samplers just before leaving. An inspection of the nets should be made at the same time to ensure that no tears are present.

Collected specimens are retained in glass vials containing 95% ethanol, which in turn are placed in a sample container also containing 95% ethanol. Before packing the sample away for the rest of the sample trip, a quick inspection of each vial should be made to assure that no more than about 50% of the vial is composed of specimens. If the volume of specimens exceeds this general guideline, the sample may not be properly preserved and can lead to deterioration though decomposition of the specimens in the sample resulting in a reduction in the accuracy of identifications. 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.

4.0 LABORATORY TECHNIQUES AND DATA INTERPRETATION

4.1 Site Identifiers

All sites sampled in or near North Carolina require a unique site identifier (SiteID). If a site has been sampled previously, use the SiteID from the prior sample. If a site meets the following criteria a new SiteID must be generated:

- is greater than one stream-mile from nearest existing site;
- has a major tributary between the sampling location and nearest existing site; and,
- has a discharge point between the sampling location and nearest existing site.

There may be other considerations for assigning new SiteIDs where precise points have not been previously sampled. Additionally, if during a special study paired samples are collected that bracket an acute point impact (such as a chemical spill), each site should be assigned a new SiteID (unless there is an existing site for one or both in the database).

4.2 Laboratory Sample Processing

When a sample is returned to the laboratory for analysis, staff identifying the sample will combine all vials collected from a site into Petri dishes for taxonomic identification. All organisms in the sample are then identified to the lowest practical taxonomic level, recorded on a Benthic Macroinvertebrate Lab Sheet (Appendix E), and tabulated as:

- Rare=1 (one or two specimens),
- Common=3 (three to nine specimens), or
- Abundant=10 (greater than nine)

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—both the portion preserved in ethyl alcohol and any microscope slides—are labeled and properly stored in the shared fish/benthos laboratory. Lab sheets and all associated information are filed by river basin, with separate file drawers for basinwide work and special studies. Collection cards are filed by CC number. Hard copies of bench sheets are filed by year, then by CC number within each year. All samples and paperwork are kept in perpetuity, barring disaster or a change to this protocol.

After the sample is identified and the lab documentation is complete, all taxonomic data, along with data from the benthos collection card and habitat assessment sheet, is entered into a Microsoft Access database. After the data are entered they are 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 are saved, Total Taxa Richness, EPT Taxa Richness, Biotic Index value, EPT Biotic Index value, and EPT abundance are automatically calculated. A species list for one or many samples can be retrieved using the database.

4.3 Bioclassifications (Ratings)

Under most conditions a bioclassification is generated following the collection of a benthic sample. Bioclassifications used by BAB to describe sites other than those at swamp waters are “Excellent,” “Good,” “Good-Fair,” “Fair,” or “Poor” for Full Scale, EPT, and Small Stream samples.

Swamp criteria use a three bioclassification approach for evaluation rather than the five classes used for other 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 (stress categories) for swamp streams are “Natural,” “Moderate,” and “Severe.”

For all samples, including swamps, two additional terms are used to indicate that no bioclassification has been generated for the sampling event: “Not Impaired” and “Not Rated.”

Further information on each of the criteria is found later in this document.

4.4 Tolerance Values

A complete list of all benthic macroinvertebrates collected is maintained in the Microsoft Access database. This index includes the taxon name, order, family, and tolerance value (an index inferring the pollution tolerance of each taxon). This list is given in Appendix E for all taxa that have been assigned a tolerance value. Tolerance values (Appendix E) were updated in April 2010 following established procedures (Lenat 1993b).

4.5 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 0.0 to 10.0, 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. A Biotic Index value 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 calculation of the Biotic Index (BI) is shown in Equation 1.

Equation 1. Biotic Index

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

Where:

B = the Biotic Index (BI)

T_i = the Tolerance Value (TV) for the i^{th} taxon

n_i = the abundance category value (1, 3, or 10) for the i^{th} taxon

N = sum of all abundance category values

4.6 Criteria for Determining Bioclassifications

North Carolina has a suite of criteria used to determine a bioclassification for a stream site. The criteria set used depends upon several factors, including: stream size, flow regime, season of collection, and sample method. Use the following key (Table 2) for direction to the appropriate assessment method.

Table 2. Site Assessment Methods

1a.	Site is located in the coastal plain, *-AND-*
	does not normally flow during a portion of the year. ..... go to 2
1b.	Site is located either in the mountains or piedmont *OR* normally flows during the entire year ..... go to 3
2a.	Swamp sample method was used, *-AND-*
	sample was collected between February 1st and March 15th ..... **evaluate using Swamp Criteria**
2b.	Not as above..... **Report bioclassification as "Not Rated"**
3a.	Site is on an unwadeable river on the coastal plain, *-AND-*
	was sampled using Boat method ..... **evaluate using Coastal B criteria but report as "Not Rated"**
3b.	Not as above..... go to 4
4a.	The site is within the Triassic Basins level 4 ecoregion *–AND-*
	the drainage area is less than 165 square miles ..... **see Triassic Basin Sites**
4b.	The site is outside of the Triassic Basin *OR* the drainage area is greater than 165 square miles ..... go to 5
5a.	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 sample method was used..... **evaluate using Small Stream criteria**
5b.	Not as above..... go to 6
6a.	Site is located in the mountains *–AND-*
	drainage area is less than 3.5 square miles *–AND-*
	site is within an undisturbed drainage *–AND-*
	EPT or Qual-4 sample method was used..... **evaluate using High Quality Small Mountain Stream criteria**
6b.	Not as above..... go to 7
7a.	Drainage area is greater than 3.0 square miles..... go to 8
7b.	Drainage area is less than or equal to 3.0 square miles..... go to 9
8a.	Full Scale sample method was used..... **evaluate using Full Scale criteria**
8b.	Either EPT or Qual-4 sample method was used ..... **evaluate using EPT criteria**
9a.	Either EPT or Qual-4 sample method was used ..... **see Unrated Small Streams**
9b.	Not as above..... **report bioclassification as "Not Rated"**

4.6.1 EPT Criteria

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

- EPT or Qual-4 sample 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; and
- the sample was not collected from the Triassic Basin level IV ecoregion.

If the sample was collected between June 1 and September 30, use Table 3. to determine 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 3. Thresholds for determining bioclassifications using EPT criteria

Bioclassification	Mountain	Piedmont	Coastal Plain
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., for those samples collected during the period of October 1 through May 31). Most insects in the orders Ephemeroptera, Plecoptera, and Trichoptera (especially those exhibiting univoltine life cycles in North Carolina) show strong seasonal patterns of development, with emergence tied to photoperiod and temperature. This means that few species in those orders with univoltine life cycles will be collected as larvae throughout any given year using the collection methods described above (adults are not collected, eggs and very small larvae pass through the sampler mesh). In North Carolina much of adult emergence, particularly among “winter” stoneflies, occurs sometime during the late winter through spring. Therefore, the greatest EPT richness occurs during the winter and spring months, with such diversity falling off into summer.

Ideally, to determine a seasonal EPT correction, 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 4).

Table 4. List of Plecoptera taxa used in seasonal adjustments.

Family	Species Name	Family	Species Name
Nemouridae		Perlodidae	
	Amphinemura spp		*Clioperla clio*
	Prostoia spp		*Cultus decisus* complex
	Shipsa rotunda		*Cultus* spp
	Soyedina spp		*Diploperla duplicata*
			Diploperla morgani
Taeniopterygidae			*Diploperla* spp
	Strophopteryx spp		*Helopicus bogaloosa*
	Taeniopteryx burksi		*Helopicus* spp
	Taeniopteryx lita		*Helopicus subvarians*
	Taeniopteryx metequi		*Isoperla burksi*
	Taeniopteryx parvulus		*Isoperla* cf *fauschi*
	Taeniopteryx spp		*Isoperla* cf *powhatan*
	Taeniopteryx ugola		*Isoperla davisi*
			Isoperla dicala
Chloroperlidae			*Isoperla frisoni*
	Alloperla spp		*Isoperla holochlora*-dark form
	Haploperla brevis		*Isoperla kirchneri* complex
	Haploperla fleeki		*Isoperla lata/pseudolata*
	Haploperla spp		*Isoperla* n sp-Collins Cr
			Isoperla n sp-Mayo R
Perlidae			*Isoperla* nr *holochlora*
	Perlinella drymo		*Isoperla* nr *transmarina*
			Isoperla orata
			Isoperla poffi
			Isoperla similis/pseudosimilis gr
			Isoperla slossonae
			Isoperla species 10
			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 determine the bioclassification for the corrected EPT in Table 3.

Note: Whether the sample method was EPT or Qual-4, enter as EPT to the BAB database. The database will correctly report the bioclassification for all EPT samples. For non-summer sampling events the seasonal taxa shown in Table 4 are automatically removed to give the adjusted EPT Richness. If a regional reference is used the number of seasonal EPT taxa may be adjusted manually in the database.

4.6.2 Full Scale criteria

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

- Full Scale sample 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; and
- Sample was not collected from the Triassic Basin level IV ecoregion.

If the sample was collected between June and September inclusive, use Table 5 to determine 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 5. Thresholds for determining BI and EPT scores using Full Scale criteria.

	Biotic Index (BI) Values			Number of EPT taxa		
Score	Mountain	Piedmont	Coastal Plain	Mountain	Piedmont	Coastal Plain
5.0	< 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
4.0	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
3.0	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
2.0	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
1.0	> 7.05	> 7.53	> 7.79	0 - 9	0 - 5	0 - 5

Seasonal corrections are required for samples collected outside of the summer sampling season (i.e., those samples collected October 1 through May 31). 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 6 for the appropriate season and level III ecoregion to the BI value as determined from the sample. Use the seasonally corrected values for BI value and EPT richness to determine the scores for each in Table 3.

Table 6. Biotic Index corrections for non-summer samples using Full Scale criteria.

Non-Summer Season	Mountain	Piedmont	Coastal Plain
Fall	+0.4	+0.1	+0.2
Winter	+0.5	+0.1	+0.2
Spring	+0.5	+0.2	+0.3

Add the BI and EPT Richness scores together and divide the result by two to get an average value. 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 (Average Score) and EPT Abundance (EPT N). Use the values in Table 7 to determine the bioclassification.

Table 7. EPT N criteria for rounding decisions using Full Scale criteria.

Bioclassification		Average Score	Mountain	Piedmont	Coastal Plain
Excellent		4.5	≥ 191	≥ 135	≥ 108
Good		4.5	≤ 190	≤ 134	≤ 107
		3.5	≥ 125	≥ 103	≥ 91
Good-Fair		3.5	≤ 124	≤ 102	≤ 90
		2.5	≥ 85	≥ 71	≥ 46
Fair		2.5	≤ 84	≤ 70	≤ 45
		1.5	≥ 45	≥ 38	≥ 18
Poor		1.5	≤ 44	≤ 37	≤ 17

Note: The BAB benthos database will correctly report the bioclassification for all Full Scale samples regardless of season. For non-summer sampling events the seasonal taxa shown in Table 4 are automatically removed to give the adjusted EPT Richness. If a regional reference is used the number of seasonal EPT taxa may be adjusted manually in the database.

4.6.3 Triassic Basin sites

Triassic stream sites with small catchments contained entirely or mostly within the Triassic Basin will not receive a bioclassification (report bioclassification as "Not Rated"). Stream watersheds overwhelmingly composed of Triassic Basin geology are frequently subject to flow cessation (even during periods of normal precipitation) and typically exhibit an acute lack of colonizable habitat suitable for aquatic invertebrates. These hydrological and physical deficiencies make interpretation of biological data difficult, and for this reason these systems are not rated. However, large waterbodies that do not originate in the Triassic Basin, and whose majority of its watershed is not composed of Triassic geologies, can be rated since these systems do not have the hydrological or habitat deficiencies common to smaller Triassic Basin waterbodies. These rateable waterbodies have drainage areas of 165 square miles or more and include Dan River, Deep River, Smith River, Mayo River, Little River, and Flat River.

For Triassic Basins sites with catchments less than 165 square miles in area and for which the catchment is mostly outside of the Triassic Basin level IV ecoregion, a judgment is made based upon the physical characteristics of the site. If the site exhibits typical Triassic Basins characteristics (i.e., lack of rocky riffles, lack of root mats, predominance of sand and clay substrate, uniform channel shape), then the site is not rated (report bioclassification as "Not Rated"). If the site is not typically Triassic, continue to couple 5 of the key to criteria (Section 4.6).

4.6.4 Small Stream Criteria

Small Stream criteria are appropriate to use when all of 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 Basin level IV ecoregion;
- Sample was collected between April 1 and June 30; and
- Qual-4 sample method was used.

Use Table 8 to determine the bioclassification based upon the NCBI value for the sample and ecoregion from which the sampled was collected (there is no seasonal adjustment). Note that these threshold values were adjusted after tolerance values were updated in 2010 and therefore differ from those in the small streams biocriteria development report (NCDWQ 2009).

Table 8. NCBI thresholds for determining bioclassifications using Small Stream criteria.

Bioclassification	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 stream sites meeting these criteria. The "Manual Assignment" box should be checked when entering data and the appropriate bioclassification (as determined above) when entered to the drop-down box in the electronic database maintained by the BAB.

4.6.5 High-Quality Small Mountain Stream criteria

High-Quality Small Mountain Stream (HQSMS) Criteria are appropriate to use when all of 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 sample method was used; and
- site cannot be evaluated using Small Stream criteria (above).

If the sample was collected outside of the summer sampling period (i.e. the sample was collected during the period of October 1 through May 31), make the seasonal adjustment as discussed under the use of EPT criteria described 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 use that value in Table 6 to determine the bioclassification for the site.

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

4.6.6 Unrated Small Streams

Apply the following rules when all of the following conditions are met:

- drainage area is less than or equal to 3.0 square miles;
- EPT or Qual-4 sample 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; and
- neither Small Stream nor HQSMS criteria can be applied.

Determine the bioclassification from Table 8 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 sample 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.

4.6.7 Coastal B (Boat) Criteria

Coastal B criteria are appropriate to use when all of 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; and
- Boat sample method was used.

A provisional bioclassification can be determined by looking up the EPT Richness for the sample in Table 9 below. When entering data to the BAB benthos database the "Manual Assignment" box must be checked and "Not Rated" selected from the drop-down box.

Table 9. EPT richness thresholds for determining provisional bioclassifications using Coastal B criteria.

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

4.6.8 Swamp Stream Criteria

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

- site is located on the coastal plain;
- there is normally little to no flow at the site during a portion of the year (typically May-September);
- collection was made between February 1 and March 15; and
- Swamp sample method was used.

In general, the BAB does not give a rating for stream sites where the pH is 4.0 or less. For most swamp regions, sites are not rated when the pH is 4.5 or less (regions A, B, P—see Table 10 below). Swamp region C is not rated if the pH is 4.1 or less (see Table 10 below).

Use the following key and the maps in Appendix F to determine the appropriate swamp region for the site:

Table 10. Swamp region classifications

1a.	Site is located in the Level IV *Chesapeake-Pamlico Lowlands and Tidal Marshes* (63b) ecoregion –AND- within HUC 03010205. See Appendix F ..... **use Swamp Region C**
1b.	Not as above..... go to 2
2a.	Site is 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 as "Not Rated"**
2b.	Not as above..... go to 3
3a.	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. See Appendix F ..... **use Swamp Region B**
3b.	Not as above..... go to 4
4a.	Site is located within the Level IV *Atlantic Southern Loam Plains* (65l) or *Rolling Coastal Plain* (65m) ecoregions. See Appendix F..... **use Swamp Region A**
4b.	Not as above..... go to 5
5a.	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. See Appendix F ..... **use Swamp Region P**
5b.	Not as above..... go to 6
6a.	Site is located within the Waccamaw River drainage. See Appendix F. ..... **use Swamp Region S**
6b.	Not as 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.

4.6.8.1 Swamp Total Taxa Richness Score

To find the Total Taxa Richness Score for the site, first determine the Corrected Total Taxa Richness for the sample:

- If the channel is braided, Corrected Total Taxa Richness is Total Taxa Richness + 8
- If the channel is **not** braided, Corrected Total Taxa Richness is Total Taxa Richness (no adjustment)

Taxa Richness Score (last row in Table 11) depends upon swamp region, pH, and Corrected Total Taxa Richness.

Table 11. Swamp Total Taxa Richness Score Lookup

pH	Corrected Total Taxa Richness		
Swamp Regions A,P,S			
≥ 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	20-37	< 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
Swamp Region B			
≥ 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	---
≤ 4.4	Not Rated	Not Rated	Not Rated
Swamp Region C			
≥ 4.1	> 34	0-34	---
≤ 4.0	Not Rated	Not Rated	Not Rated
Swamp Total Taxa Richness Score (T):	5	3	1

4.6.8.2 Swamp Biotic Index Score

Use Table 12 to determine the Biotic Index Score (B) from the swamp region and the Biotic Index for the site.

Table 12. Swamp 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
Swamp Biotic Index Score (B):	5	3	1

4.6.8.3 Swamp EPT Richness Score

To find the Total Taxa Richness Score for the site, first determine the Corrected EPT Richness for the sample:

- If the channel is braided, Corrected EPT Richness is EPT Richness + 2
- If the channel is **not** braided, Corrected EPT Richness is EPT Richness (no adjustment)

Use **Error! Reference source not found.**Table 13 to determine the EPT Taxa Richness Score for swamp r egions A, P, S, and B. 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 Taxa Richness.

Table 13. Swamp EPT Taxa Richness Score lookup for Swamp Regions A, P, S and B

pH	Corrected EPT Richness		
Swamp Regions A or P			
≥ 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	0 - 4	---
≤ 4.4	Not Rated	Not Rated	Not Rated
Swamp Region S			
≥ 4.1	> 10	6 - 10	0 - 5
≤ 4.0	Not Rated	Not Rated	Not Rated
Swamp Region B			
≥ 4.1	> 4	2 - 4	0 - 1
≤ 4.0	Not Rated	Not Rated	Not Rated
Swamp EPT Taxa Richness Score (E):	5	3	1

4.6.8.4 Swamp Habitat Score

Use Table 14 to determine the Habitat Score from the final value on the Habitat Assessment Field Data Sheet (Appendix A).

Table 14. Swamp habitat score lookup for all regions.

Habitat Value	Swamp Habitat Score (H)
< 60	1
60 - 79	3
> 79	5

4.6.8.5 Swamp Site Score and Bioclassification determination

Use Equation 2 to calculate the final swamp site score for swamp regions A, B, P or S, or use Equation 3 to calculate the swamp score for swamp region C.

Equation 2. Final Swamp Site Score for Swamp Regions A, B, P or S

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

Equation 3. Final Swamp Site Score for Swamp Region C

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

Where:

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

Table 15. Swamp Bioclassification Lookup

Final Swamp Site Score	Swamp Bioclassification
0-3	Severe
4-8	Moderate
9-10	Natural

Use **Error! Reference source not found.**Table 15 to determine the swamp bioclassification for the site using t he Final Swamp 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 menu.

4.7 Midge Deformity Analysis

When a waterbody is impacted by organic compounds as well as toxic chemicals, the resulting benthic community is frequently dominated by typical organic indicator taxa, with midges in the genus *Chironomus* among those taxa. Many studies have shown an association between deformities in chironomid larvae and the presence of environmental stressors (e.g., Hamilton and Saether 1971, Wiederholm 1984, Dermott 1991, Janssens de Bisthoven 1992, Madden et al. 1992, Bird 1994, Hudson and Ciborowski 1996a, Hudson and Ciborowski 1996b, Diggins and Stewart 1998, Janssens de Bisthoven and Ollevier 1998, Warwick and Tisdale 1988, Martinez et al. 2001, Martinez et al. 2002, Swansburg et al. 2002, MacDonald and Taylor 2006). Using larvae from DWR benthic samples and toxicity data from the Aquatic Toxicology Branch, relationships were found between toxicity and *Chironomus* mentum deformities (Lenat 1993a), leading to the use of the organism as a screening tool for toxicity. As stated in the abstract to Lenat's 1993 publication:

> The percentage of deformities in *Chironomus* menta was compared with both an independent measure of stream water quality and predictions of instream toxicity. Simple organic loading caused small (nonsignificant) increases in the frequency of deformities, whereas toxic conditions caused large increases in both the number and types of deformities.

At least 20 *Chironomus* head capsules should be slide-mounted from any site to be screened. Each mentum is visually analyzed and 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 score is computed for each site that gives greater weight to more severe deformities:

Equation 4. Toxicity Score

$$S = \frac{(C_1 + 2C_2 + 3C_3) * 100}{n}$$

Where:

S = toxicity score

C_1, C_2, and C_3 are the number of Class I, Class II, and Class III deformities, respectively

n = total number of larvae used in the analysis

No significant between-group differences were found for nontoxic sites with bioclassifications of Excellent, Good, and Good-Fair. The percent deformities for these unpolluted sites averaged about 5%, with a mean toxicity score of about 7. Nontoxic sites with Fair and Poor bioclassifications are combined into a polluted/nontoxic group, with a deformity rate of 12% and a mean toxicity 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 toxicity score of 52. A significant increase was seen for the Poor/Toxic group: mean deformity rate = 45%, mean toxicity score = 100. Both toxic groups also are characterized by a high proportion of Class II and Class III deformities.

5.0 QUALITY ASSURANCE

DWR complies with all USEPA requirements regarding competency of field staff, laboratory procedures, and other quality assurance measures as described in the approved Quality Management Plan (2010).

Quality assurance begins with following the procedures found in this manual, as well as 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 each day, and 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[4] 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 on the BAB network drive. Taxonomic assistance is obtained from specialists when appropriate. Reference specimens 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 undergoing identification and enumeration 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 16. If a single error (e.g., the misidentification of a specimen) has more than one consequence (e.g., Misidentification *and* Abundance Classification errors), then only the error with the highest point deduction is counted against the original taxonomist. The final score is determined as follows, with a score of less than 90 considered a fail:

Equation 5. Quality Assurance Score:

$$S = \left(1 - \frac{E}{T}\right) \times 100$$

Where:

S = the QA Score
E = the sum of the error points
T = the number of taxa present in the sample (as determined by the QA biologist

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

Table 16. Errors and associated points for taxonomic quality assurance checks.

Error Description	Point Deduction Per Error	Error Example
Misidentification	1	*Baetis pluto* is the "correct identification" (i.e. an identification agreed upon by two or more BAB biologists). 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.

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6.0 REFERENCES

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

Bird, G. A. 1994. Use of chironomid deformities to assess environmental degradation in the Yamaska River, Quebec. Environmental Monitoring and Assessment 30:163–175.

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. Journal of the North American Benthological Society 13:30-44.

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

Dermott, R.M. 1991. Deformities in larval *Procladius* spp. and dominant Chironomini from the St. Clair River. Hydrobiologia 219:171-185.

Diggins, T.P., and Stewart, K.M. 1998. Chironomid deformities, benthic community composition, and trace elements in the Buffalo River (New York) Area of Concern. Journal of the North American Benthological Society 17:311-323.

Eaton, L. E. and 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. and 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.

Hamilton A. L. and Sæther, O. A. 1971. The occurrence of characteristic deformities in the chironomid larvae of several Canadian Lakes. Canadian Entomologist 103:363 - 368.

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

Hudson, L.A., and J.J.H. Ciborowski. 1996a. Spatial and taxonomic variation in incidence of mouthpart deformities in midge larvae (Diptera: Chironomidae: Chironomini). Canadian Journal of Fisheries and Aquatic Sciences 53:297-304.

Hudson, L.A, and J.J.H. Ciborowski. 1996b. Teratogenic and genotoxic responses of larval Chironomous salinarious group (Diptera: Chironomidae) to contaminated sediment. Environmental Toxicology and Chemistry 15:1375-1381.

Janssens de Bisthoven, L., K. R. Thimmermans, and F. Ollevier. 1992. The concentration of cadmium, lead, copper and zinc in *Chironomus* gr *thummi* larvae (Diptera: Chironomidae) with deformed versus normal menta. Hydrobiologia 239:141–149.

Janssens de Bisthoven, L., A. Vermeulen and F. Ollevier. 1998. Experimental induction of morphological deformities in *Chironomus riparius* larvae by chronic exposure to copper and lead. Archives of Environmental Contamination and Toxicology 35:249–256.

Larsen, D. P. and A.T. Herlihy. 1998. The dilemma of sampling streams for macroinvertebrate richness. Journal of the North American Benthological Society 17: 359-366.

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.

Lenat, D.R. 1993a. Using mentum deformities of *Chironomus* larvae to evaluate the effects of toxicity and organic loading in streams. Journal of the North American Benthological Society 12: 265-269.

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

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 20: 287-298.

MacDonald, E.E. and B.R. Taylor. 2006. Incidence of mentum deformities in midge larvae (Diptera: Chironomidae) from Northern Nova Scotia, Canada. Hydrobiologia 563:277-287.

Madden, C. P., P. J. Suter, B. C. Nicholas, and A. D. Austin. 1992. Deformities in chironomid larvae as indicator of pollution (pesticide) stress. Netherlands Journal of Aquatic Ecology 26:551–557.

Martinez, E.A., B.C. Moore, J. Schaumloffel & N. Dasgupta. 2001. Induction of morphological deformities in *Chironomus tentans* exposed to zinc- and lead- spiked sediments. Environmental Toxicology and Chemistry 20:2475–2481.

Martinez, E.A., B.C. Moore, J. Schaumoloffel, and N. Dasgupta. 2002. The potential association between menta deformities and trace elements in Chironomidae (Diptera) taken from a heavy metal contaminated river. Archives of Environmental Contamination and Toxicology 42:286–291.

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. Freshwater Invertebrate Biology 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. Journal of the Water Pollution Control Federation 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.

Swansburg, E.O., W.L. Fairchild, B.J. Fryer, and J.J.H. Ciborowski. 2002. Mouthpart deformities and community composition of Chironomidae (Diptera) larvae downstream of metal mines in New Brunswick, Canada. Environmental Toxicology and Chemistry 21: 2675–2684.

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

Warwick, W.F., and N.A. Tisdale. 1988. Morphological deformities in *Chironomus*, *Cryptochironomus*, and *Procladius* larvae (Diptera: Chironomidae) from two differentially stressed sites in Tobin Lake, Saskatchewan. Canadian Journal of Fisheries and Aquatic Sciences 45:1123-1144.

Wiederholm, T. 1984. Incidence of deformed chironomid larvae (Diptera: Chironomidae) in Swedish lakes. Hydrobiologia 109:243-249

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, and C.E. Cushing. 1980. The river continuum concept. Canadian Journal of Fisheries and Aquatic Sciences 37:130-137.

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:_____
_____

_____

_____

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.

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

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	Lft. Bank Score	Rt. Bank Score
Dominant vegetation: ☐ Trees ☐ Shrubs ☐ Grasses ☐ Weeds/old field ☐Exotics (kudzu, etc)		
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

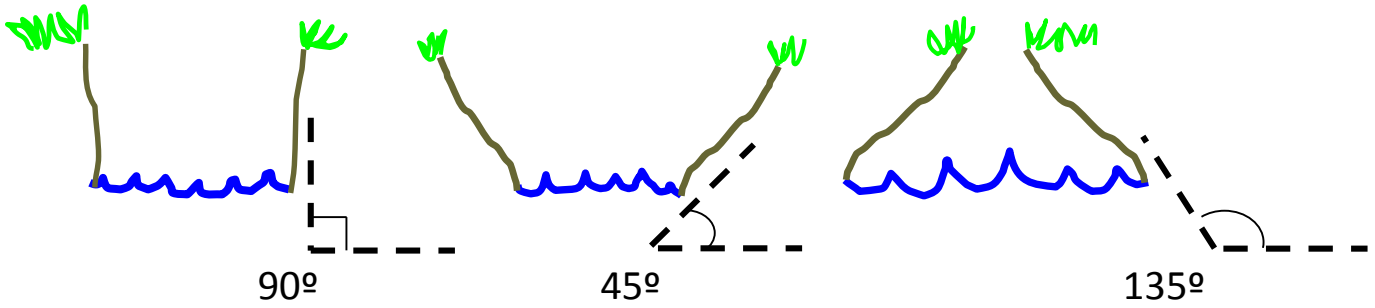
Remarks_____ Subtotal_____

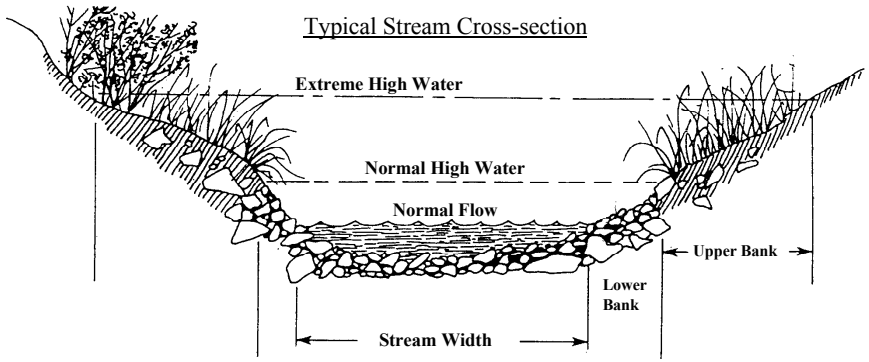
Page Total_____

☐ 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..... ☐

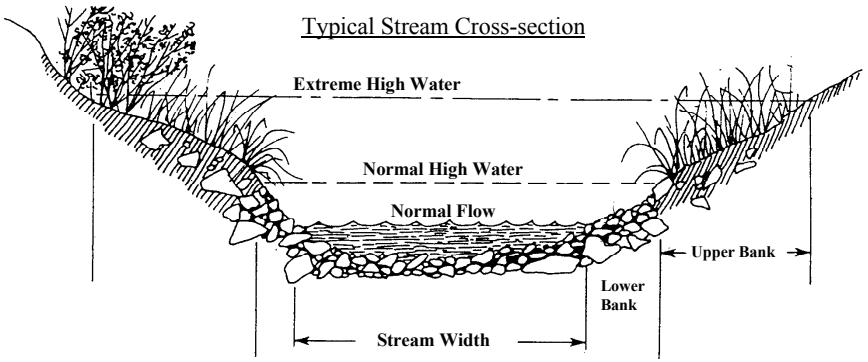
Turbidity: ☐Clear ☐ Slightly Turbid ☐Turbid ☐Tannic ☐Milky ☐Colored (from dyes) ☐Green tinge
Good potential for Wetlands Restoration Project?? ☐ YES ☐ NO Details_____
_____

☐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



This side is 45º bank angle.

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 _____

BENTHOS COLLECTION CARD

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

WATERBODY _____

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

Substrate:

Boulder (10")	____	%
Cobble (2 1/2-10")	____	%
Gravel (1/12-2 1/2")	____	%
Sand (1/12")	____	%
Silt, fine Partic.	____	%
Other	____	%

River:

Mean depth	____
Maxim. depth	____
Width	____
Current	____
Recent Rain ?	____

Photos (#) _____

Field Parameters:

Bank Erosion	N ____	Mod ____	Sev ____		
Canopy	% ____	Type ____			
Aufwuchs	N ____	Mod ____	Abund. ____		
Podostemum	N ____	Mod ____	Abund. ____		
Tribs Present?	____				

Instream Habitat: (0,+,++)

Pools	____	Backwaters	____
Riffles	____	Detritus	____
Snags	____	Aquatic Weeds	____
Undercut Banks	____	Other	____
Root Mats	____		

Samples: (#)

Kicks	____
Sweeps	____
Leaf Packs	____
Rock-Log	____
Sand	____
Visuals	____
Other	____

Water Chemistry:

Temperature (°C)	____
Dissolved Oxygen (mg/L)	____
Conductivity (µmhos/cm)	____
pH	____

Field Observations: _____

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.

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 gr	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
		Labiobaetis ephippiatus	3.5
		Labiobaetis frondalis	4.6
		Labiobaetis propinquus	5.8
		Paracloeodes spp	8.0
		Plauditus cestus	4.6
		Plauditus dubius gr	2.2
		Procloeon spp	1.9
	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

Order	Family	Latin Name	Tolerance Value
		Ephemerella hispida	0.1
		Ephemerella invaria gr	2.6
		Ephemerella rossi gr	0.0
		Ephemerella rotunda	1.8
		Ephemerella spp	2.1
		Eurylophella bicolor	4.8
		Eurylophella doris	7.0
		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
		Teloganopsis 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 gr	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

Order	Family	Latin Name	Tolerance Value
		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
		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

Order	Family	Latin Name	Tolerance Value
		Isoperla davisi/nr transmarina	4.8
		Isoperla holochlora-dark form/cf powhatan	1.2
		Isoperla holochlora-light form	0.7
		Isoperla kirchneri complex	2.5
		Isoperla nr holochlora	0.0
		Isoperla orata	0.0
		Isoperla poffi/n sp-Collins Cr	5.2
		Isoperla similis/pseudosimilis gr	0.8
		Isoperla spp	3.2
		Malirekus hastatus	1.0
		Remenus spp	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
		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
		Cheumatopsyche spp	6.6
		Diplectrona modesta	2.3
		Hydropsyche (C.) alhedra	0.0
		Hydropsyche (C.) bronta	2.3

Order	Family	Latin Name	Tolerance Value
		Hydropsyche (C.) macleodi	0.7
		Hydropsyche (C.) morosa	2.3
		Hydropsyche (C.) slossonae	0.0
		Hydropsyche (C.) sparna	2.5
		Hydropsyche (H.) betteni	7.9
		Hydropsyche (H.) decalda	3.2
		Hydropsyche (H.) demora	2.6
		Hydropsyche (H.) incommoda	4.6
		Hydropsyche (H.) phalerata	3.7
		Hydropsyche (H.) rossi	4.8
		Hydropsyche (H.) scalaris	2.6
		Hydropsyche (H.) 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
		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

Order	Family	Latin Name	Tolerance Value
		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 sensu lato spp	3.1
	PSYCHOMYIIDAE	*Lype diversa*	3.9
		Psychomyia flavida	3.0
		Psychomyia nomada	2.0
	RHYACOPHILIDAE	*Rhyacophila acutiloba*	0.0
		Rhyacophila appalachia/nigrita	0.0
		Rhyacophila atrata	0.0
		Rhyacophila carolina	0.4
		Rhyacophila fenestra/ledra	4.6
		Rhyacophila formosa	0.1
		Rhyacophila fuscula	1.6
		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
	CORDULEGASTRIDAE	*Cordulegaster spp*	5.7
	CORDULIIDAE	*Epitheca princeps*	7.3

Order	Family	Latin Name	Tolerance Value
		Epitheca spp	8.0
		Helocordulia spp	5.8
		Neurocordulia obsoleta	5.3
		Neurocordulia spp	5.3
		Neurocordulia virginiensis	1.1
		Somatochlora spp	8.9
	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 spp	8.2
		Stylogomphus albistylus/sigmastylus	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

Order	Family	Latin Name	Tolerance Value
		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
		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

Order	Family	Latin Name	Tolerance Value
		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
		Kribiodorum perpulchrum	4.0
		Labrundinia pilosella	6.2
		Labrundinia spp	6.2
		Larsia spp	6.5
		Lopescladius spp	1.2
		Micropsectra spp	2.4
		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

Order	Family	Latin Name	Tolerance Value
		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
		Robackia claviger	1.9
		Robackia demeijerei	4.3
		Saetheria tylus	7.3
		Stempellinella spp	5.6
		Stenochironomus spp	6.3
		Stictochironomus spp	5.4
		Sublettea coffmani	1.4
		Sympotthastia spp	4.5

Order	Family	Latin Name	Tolerance Value
		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
		Thienemanniella spp	6.4
		Thienemanniella 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 spp	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

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
		Menetus 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

Order	Family	Latin Name	Tolerance Value
		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 graecense*	6.6

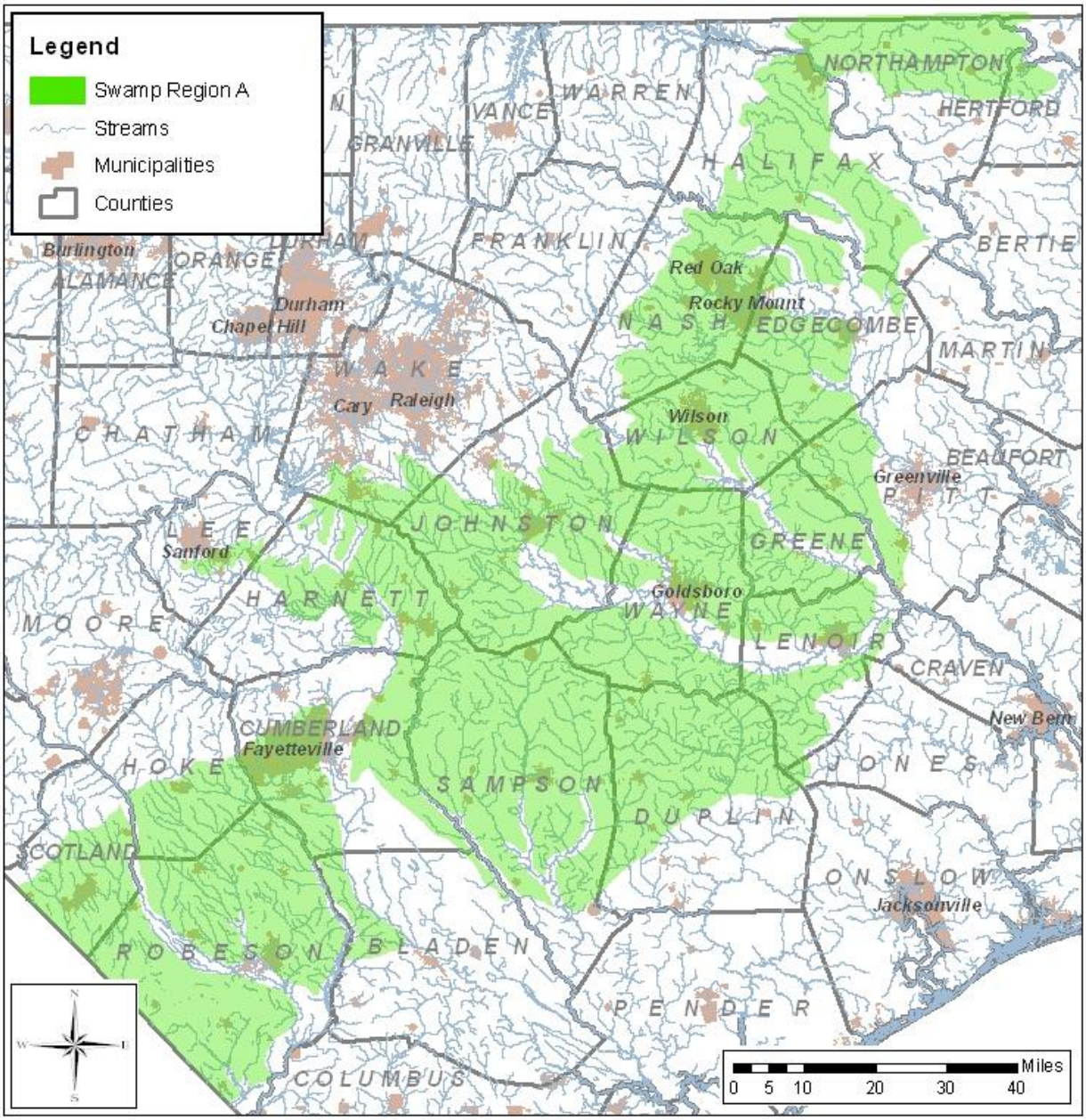


Figure F1. Swamp Region A

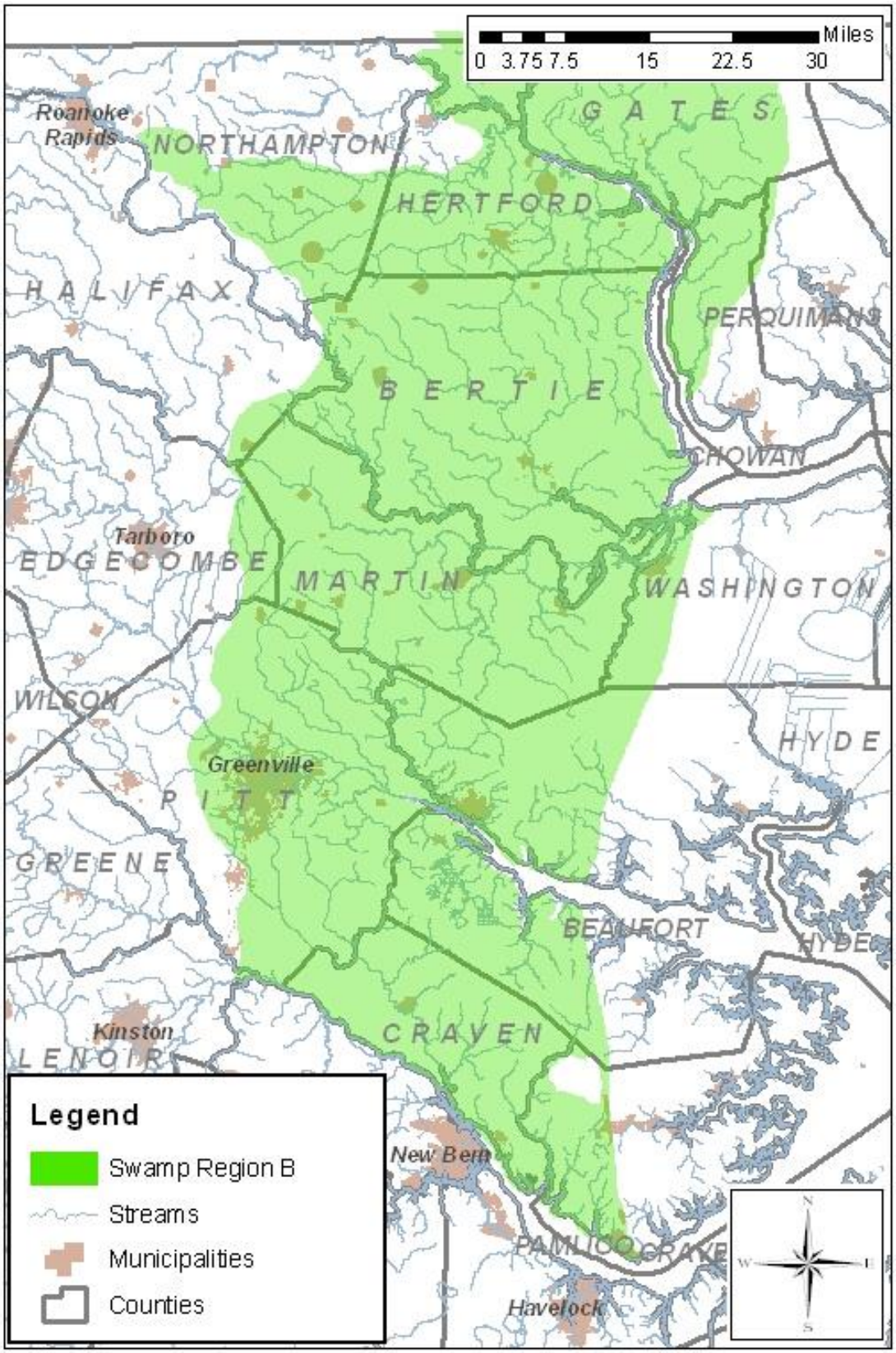


Figure F2. Swamp Region B.

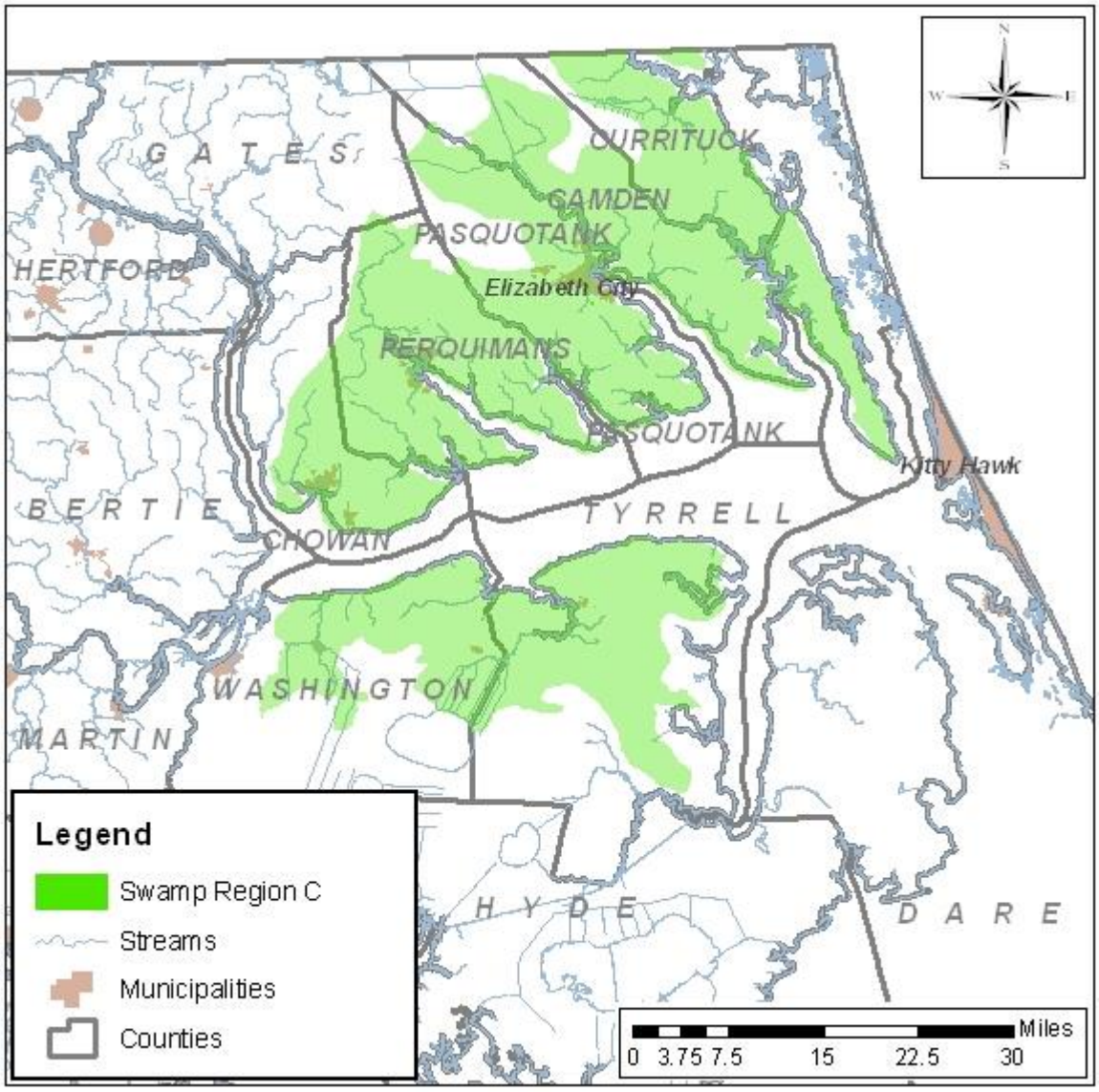


Figure F3. Swamp Region C.

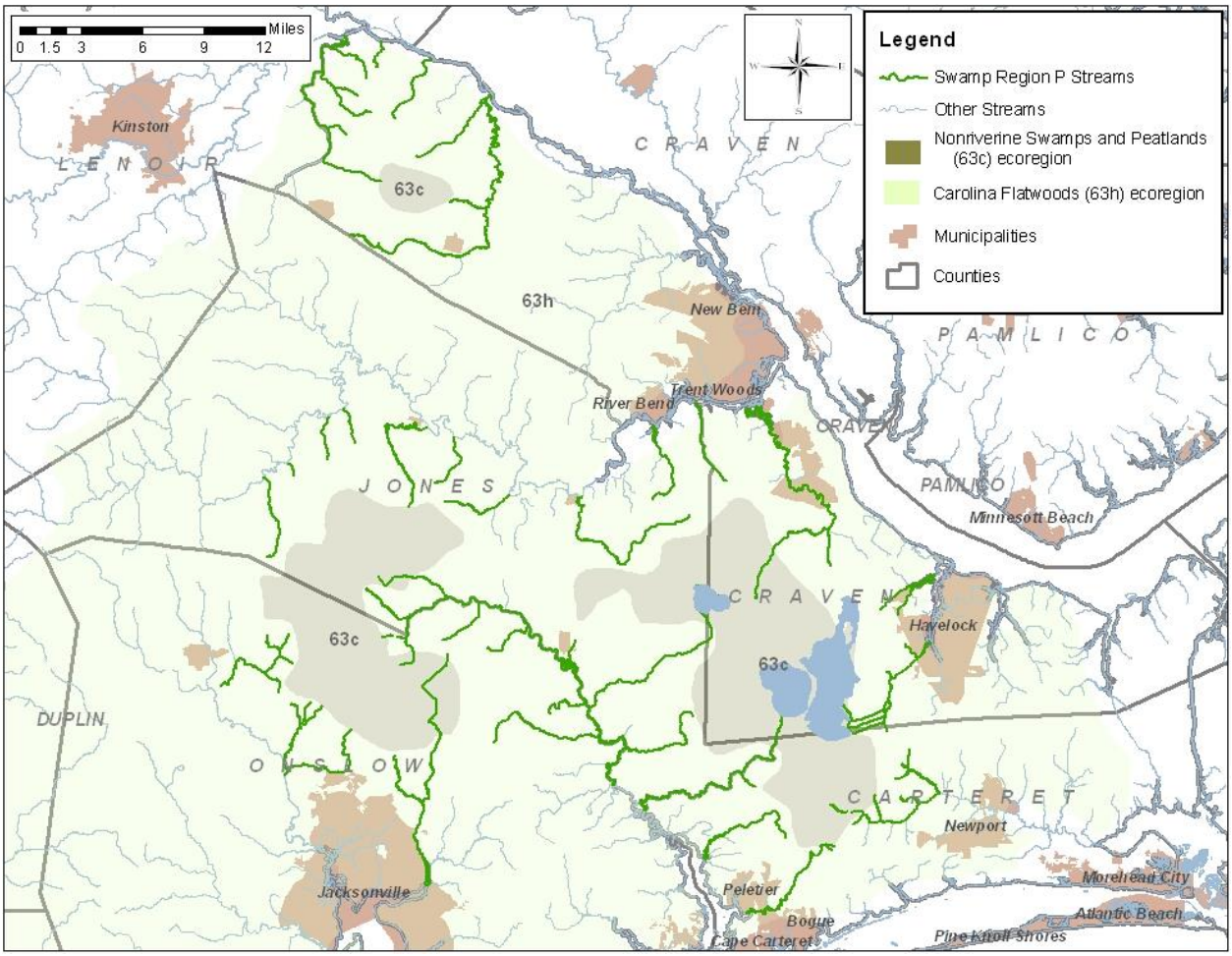


Figure F4. Northern portion of Swamp Region P.

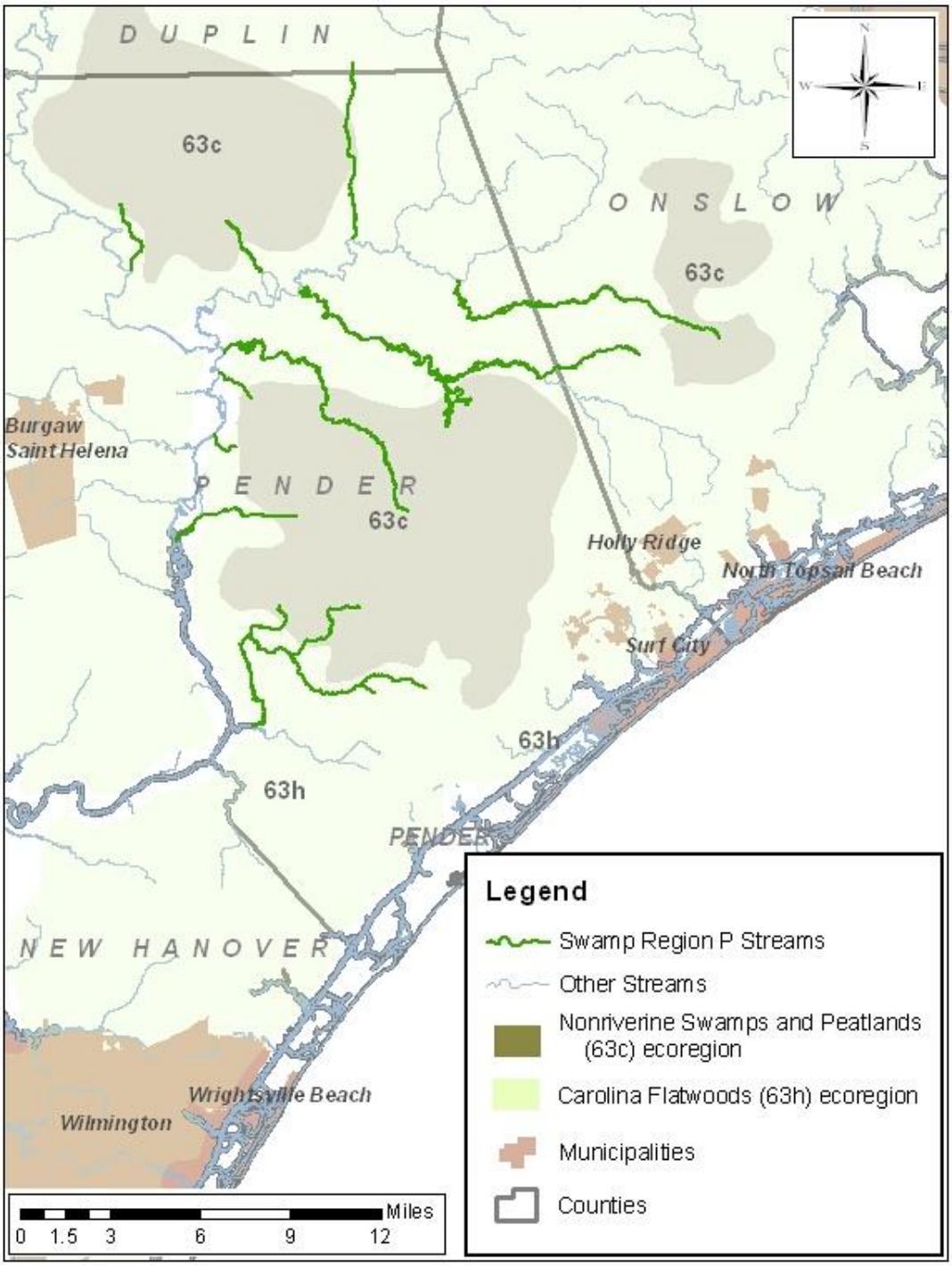


Figure F5. Middle portion of Swamp Region P.

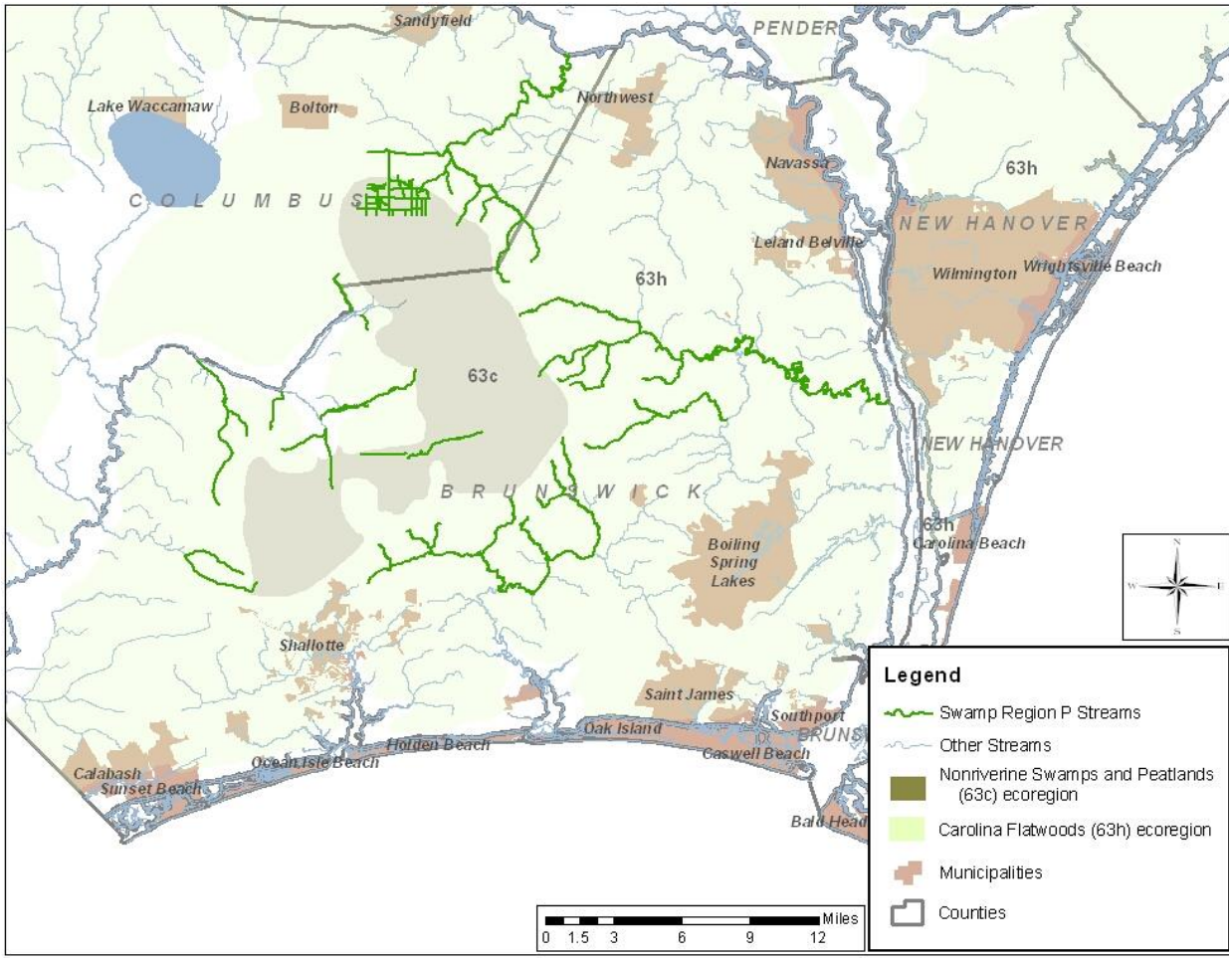


Figure F6. Southern portion of Swamp Region P.

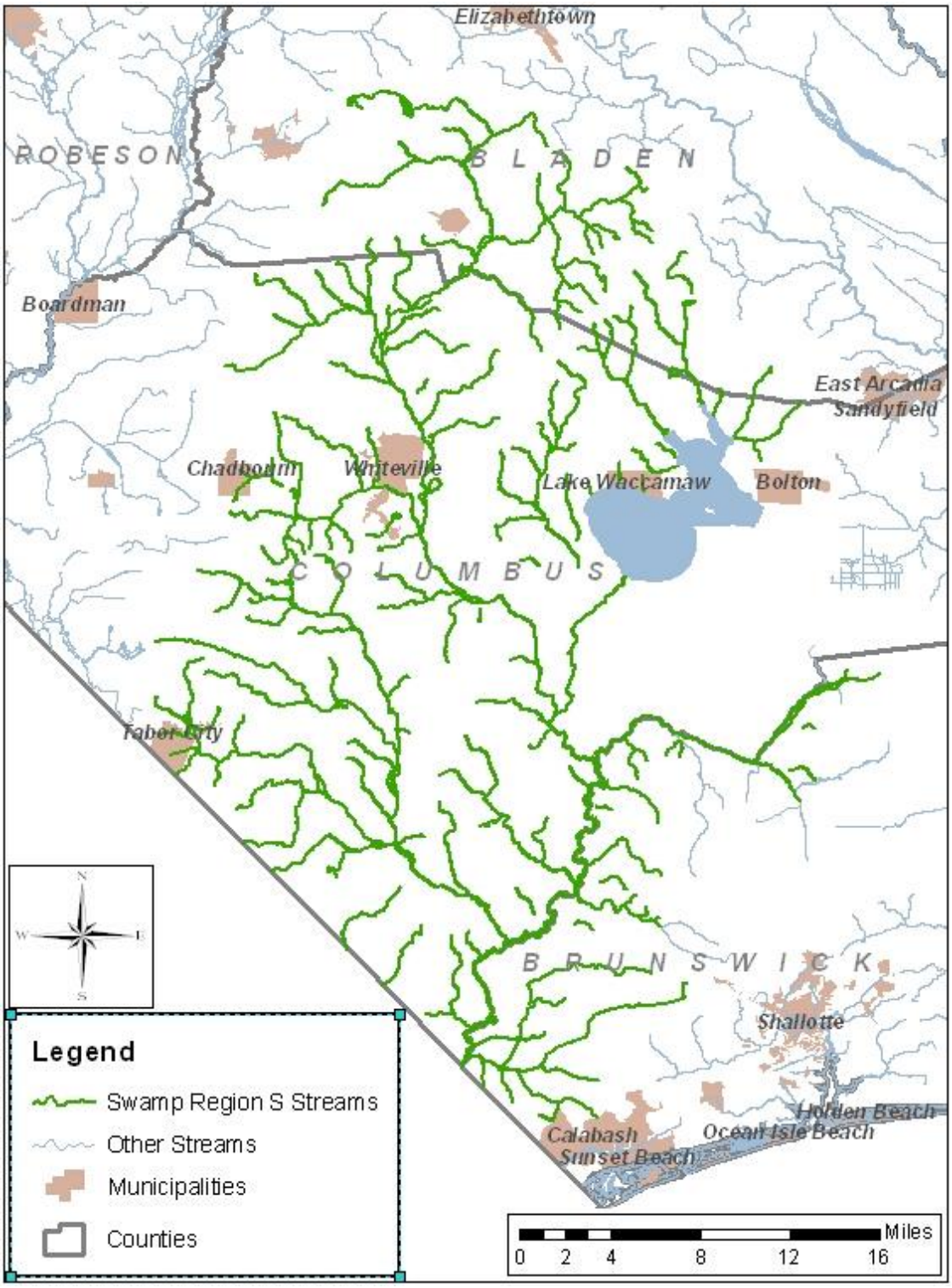


Figure F7. Swamp Region S