Freshwater Macroinvertebrates Survey Protocols

Project Development and Environmental Analysis Branch		Approved: 3/26/2009 Version 1.0

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Purpose

The purpose of this procedure is to give guidance on sampling for macroinvertebrates in streams that may be impacted by NCDOT projects. The procedure outlines sampling requirements and techniques.

Responsibility

The NEU Biological Surveys Group (NEU-BSG) is responsible for conducting surveys for macroinvertebrates. The request is made from the NEU Environmental Specialist assigned to the project if a survey is required.

Scheduling and Time Constraints

None

Procedures

Sampling Requirements	General Field Procedures	Sampling Methodologies
Sampling Techniques	Laboratory Techniques /Data Interpretation	

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Procedure 1: Sampling Requirements

The following are guidelines to use to determine whether a stream survey should be conducted.

Step 1. Determine the current condition of stream flow and prior flow conditions if possible by looking up current data from USGS gage stations. The sampling methodologies described in this procedure require that freshwater streams or rivers be wadeable for efficient data collection.

Step 2. If high water conditions are encountered, it is better to return to the site during a more appropriate flow regime.

High water conditions severely impair sampling efficiency by making some critical habitats inaccessible. An underestimate of taxa richness due to high flows may lead to an incorrect assessment of water quality.

Step 3. Drought conditions also alter the composition of benthic fauna. Below is information on dealing with drought conditions.

- Every effort should be made to insure that flow has been continuous prior to sampling, especially in areas of the state prone to drought conditions. 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, if prior flow conditions have been extreme-either high or low.
- Use the Internet to check stream stage height from the closest USGS gage station before traveling to the site.

Step 4. An experienced benthic biologist trained and skilled in field benthic sampling methods and organism identification must be present for all sample collections. New or inexperienced personnel can be used as team members, if close supervision is provided by the experienced biologist during sample collection, during sample picking (look through trays again before discarding), and during visuals.

Procedure 2: General Field Procedures

Follow the steps below for documenting field observations and data recording

Step 1. Immediate watershed

- Type of land use
- Extent of disturbed land
- Any floodplain deposition of sediment
- Any evidence of stream widening and/or filling in
- Presence of upstream tributaries or dams (including beaver dams)
- Evidence of recent water level changes such as leaf packs out of water
- Submerged terrestrial vegetation
- Sediment on vegetation above water level
- Any livestock with access to stream

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- Any point sources
- Any unique habitats.

Step 2. Substrate

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

Step 3. Width

- 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 whole number.
- Any unusual characteristics, such as a braided channel in coastal areas, should be noted and recorded.

Step 4. Water

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

Step 5. Benthic Community

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

Step 6. Samples are labeled before leaving the site with the following information:

- Collection site and station,
- Sample ID#
- Initials of collectors
- Date of collection.
- Water temperature
- pH
- Conductivity
- Dissolved oxygen measurements

Step 7. Take a gage reading if one is present and take photographs of the site.

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Procedure 3: Sampling Methodologies

The NCDWQ Biological Assessment Unit uses four different macroinvertebrate collection methods.

Step 1. Standard Qualitative Method

The Standard Qualitative Method can be used to assign water quality ratings to most wadeable, flowing streams and rivers in North Carolina. It is applicable for most between-site and/or between date comparisons, and is used by the DWQ for all evaluations of impaired streams (those on the 303d list), that are large enough to be assigned a bioclassification.

This method consists of two kick net samples, three sweep-net samples, one leaf pack sample, two fine mesh rock/log wash samples, one sand sample, and visual collections. This method is used by the NCDOT when a more comprehensive type of study is conducted.

Invertebrates are removed form the sample in the field (picked) using forceps and white plastic shallow trays, and preserved in glass vials containing 95% ethanol.

Organisms are picked roughly in proportion to their abundance in the sample. It is not intended to remove all organisms. If an organism can be reliably identified in the field, then no more than 10 individuals need to be collected.

Step 2. EPT method

The EPT method is an abbreviated version of the regular qualitative technique. This method is used to quickly determine between site differences in water quality. This method consists of one kick, one sweep, one leaf pack sample and visuals.

Although the EPT method is a more rapid sampling technique, there are situations where this method may not provide enough information for an adequate assessment of water quality. Such as:

- areas with naturally low EPT richness
- areas where the abundance of more tolerant groups must be assessed.

If a biotic index is to be calculated, then the EPT method is not appropriate.

Step 3. Qual 5 Method

The Qual 5 Method uses the same collection techniques as the EPT method, with the addition of one rock/log wash. All organisms are picked from the sample, not just the EPTs. This method is faster than the standard qualitative method, and has the addition of the rock/log wash to collect the more tolerant groups. NCDOT uses this method most of the time when assessing site differences upstream and downstream of bridges. The data is used to compare site differences in the same stream, but not assign a bioclassification to the site.

Step 4. Swamp Method

Swamp streams are defined as those streams that are in the coastal plain ecoregion and that normally have no visible flow during a part of the year. This low flow period usually occurs during the summer months, but flowing water should be present in swamp streams during the winter months. So these streams are sampled in the winter (February to early March) allowing for the best opportunity for detecting differences in communities from what is natural.

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The swamp sampling method utilizes a variety of collection techniques to inventory the macroinvertebrate community. A total of nine sweep samples (three by each field team member) are collected from the following habitat types: macrophytes, root mats/undercut banks, and detritus deposits. If one of these types is not present, a sweep from one of the other types is substituted. Each sweep should be emptied into a tub before another sweep is collected. Three log/debris washes and visual collections are also conducted.

Procedure 4: Sampling Techniques

Sampling techniques described below taken from NCDENR, DWQ protocols (NCDENR, 2003).

Step 1. Kick Net

A kick net is an easily constructed and versatile sampling device. It consists of a double layer of flexible nylon door or window screening held in place between two halves of a wooden pole using wood screws. The screening is reinforced with denim along all edges and has lead weights sewn into the bottom edge. The screening can be sewn onto the denim using a heavy duty sewing machine.

The net is positioned upright on the stream bed, while the area upstream is physically disrupted using feet and/or hands. The debris and organisms in the kick net are then washed down into a sieve bucket with a US Standard No. 30 mesh (0.600 mm opening) bottom, and larger leaves and debris are removed. If too coarse a mesh is used for the kick net, many animals will not be retained. If too fine a mesh is employed, the net clogs easily and washout becomes a problem. The double layer of screening works well in this respect.

Two kicks are taken from riffle areas. The two samples should be collected from areas of differing current speed. In very small streams, or in sandy areas lacking riffles, kicks should be taken from root masses, snags, or bank areas. All types of benthic macroinvertebrates are collected by this sampling device, but emphasis is placed on Ephemeroptera, Plecoptera and Trichoptera.

Step 2. Sweep Net

A long-handled triangular sweep net is another versatile sampling device. Samples are taken by physically disrupting an area and then vigorously sweeping through the disturbed area. Sweeps are usually taken from bank areas, including mud banks and root masses, and macrophyte beds. Bank samples are particularly important for the collection of "edge" species that prefer low current environments. Look for Chironomini (red chironomids), Oligochaeta, Odonata, mobile cased

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Trichoptera, *Sialis*, Crustacea, and certain Ephemeroptera. A sweep net also can be used to sample gravel riffle areas where stone-cased Trichoptera may be abundant.

Step 3. Fine Mesh Sampler

Since the kick and sweep nets utilize a relatively coarse mesh size, an alternate sampling technique was devised to sample the smaller invertebrates (especially the Chironomidae). The resulting sampler is known as a "chironomid-getter". Fine nitex mesh (300 microns) is placed between four inch PVC pipe fittings that are designed to screw together. The exact dimensions are not critical, but the cylinder should be able to fit inside another container, usually a slightly larger, round plastic container. This device can be used in a variety of ways.

The simplest technique is to wash down rocks or logs in a large plastic tub partially filled with water. Rocks are selected which have visible growths of periphyton, Podostemum, or moss. Any large particulate material (leaves, etc.) is washed down and discarded. A single composite sample can be made from several (usually 10-15) rocks and/or logs. The material remaining in the tub is poured through the fine mesh sampler and the water allowed to drain out completely.

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The residue is preserved in 95% ethanol. This is accomplished by placing the fine mesh sampler into another container (6 cup size round plastic food storage container works well) which is half filled with alcohol.

The sample is allowed to sit for several minutes, pulled out of the alcohol, and then backwashed into a picking tray. This method of field preservation requires only a small amount of alcohol, and it may be reused several times. Take care to rinse samplers between sites.

Field preservation makes small chironomids and oligochaetes more visible, and easier to pick up with forceps. This technique is also good for fast moving organisms such as baetid mayflies or amphipods, or small grazing taxa such as hydroptilid caddisflies. The "pour-and-preserve" technique also can be used in conjunction with other sampling methods. For example, the elutriate from a kick or sweep sample can be processed in this manner.

Step 4. Leaf-Pack Sample

Leaf-packs, sticks and small logs are washed down in a sieve bucket with a U.S. Standard No. 30 sieve (0.600 mm openings) bottom, and then discarded. Generally, three to four leaf packs are collected from rocks or snags in fast current

areas. The best leaf packs consist of older leaves (not freshly fallen) that have begun to decay. Piles of leaves in pool areas should not be collected. Leaf-pack and small log samples are particularly useful in large sandy rivers. In such habitats, many of the species are confined to "snags" (Benke et al. 1984, Neuswanger et al. 1982). Look for "shredders", especially Tipulidae, Plecoptera, and Trichoptera.

Step 5. Visual Search

Visual inspection of large rocks and logs (the larger, the better) often adds to the species list. Large rocks and logs are a preferred microhabitat because of their stability during floods. Always look in a number of different areas (not just riffles).

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Rocks and logs in pools often yield additional species, as this habitat is not well sampled by either kicks or sweeps.

The top of rocks is a specialized microhabitat with a number of characteristic taxa. Both of the caddisflies, *Psychomyia* and *Leucotrichia,* and the lepidoptera family Pyralidae, build retreats on the top of rocks. These are often made more visible by lightly washing off any silt that has accumulated on the top of the rock. Stone cased caddisflies, such as *Glossosoma*, *Agapetus*, *Ceraclea*, and *Goera* can also be found on the top or sides of rocks. Decaying logs should be picked apart to look for chironomids, and many taxa can be found under loose bark. Rocks near the shore (in negligible current) will harbor taxa such as *Stenacron* and *Pycnopsyche*, and leaves near the shore may be the primary habitat for some Gastropoda.

Certain caddisflies (*Nyctiophylax* and related genera) select crevices in rocks or logs, often along the edge, and cover them over with silk strands. The silk becomes covered with silt and periphyton and is hard to see. There is usually a faint opening on each end of this retreat. If the tip of forceps is inserted into one opening, the larvae usually will come out the other opening. Microcaddisflies make small (2-4 millimeters) cases found attached to rocks and logs, usually on the top or along an edge. The sides of rocks are the best place to look for the caddisflies *Neophylax*, *Psilotreta* and *Agarodes*.

Polycentropodid caddisflies build funnel-shaped silken retreats (up to six inches in length) in areas of relatively slow current. Out of water, the case collapses and resembles a gelatinous brown glob. The larvae will often crawl out if left out of the water for several minutes. It's a good idea to recheck some logs during visuals for these caddisflies.

In sandy coastal plain rivers, look for a log that is in an area of faster current, with some portion raised above the substrate. This is a good place to look for hydropsychids and other filter feeders. The net may be the only visible evidence of these organisms, and they must be dug out of their retreats with forceps. Aquatic macrophytes and sponges are other habitats to be closely examined.

Approximately 10 minutes is allocated for these visual searches. In general, look for attached cases of Trichoptera, for Turbellaria (flatworms), Coleoptera (beetles), Odonata (dragonflies, especially on large logs), Gastropoda (snails), Hirudinea (leeches) and Megaloptera.

Step 6. Insure the quality of every survey by following the instructions above or noting any changes from the methodologies. It also involves taking care of equipment. Equipment care includes, but is not limited to:

- Inspecting nets for holes both before and after sampling
- Rinsing all nets and tubs carefully between sites and upon returning from the field
- Calibrate meters both before and after use when called for in the meter's operating instructions

Procedure 5: Laboratory Techniques and Data Interpretation

Step 1. Return vial samples to the laboratory for analysis.

Step 2. The person identifying the sample will combine all vials collected from a site into one petri dish for identification.

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Step 3. Identify all organisms in the sample to the lowest possible taxonomic level and record on a Benthic Macroinvertebrate Lab Sheet. (future link). Tabulate the observed organisms as

- Rare (1-2 specimens),
- Common (3-9 specimens)
- Abundant ($\geq$10 specimens).

Most organisms may be identified using only a dissecting microscope, but Oligochaeta, Chironomidae and many polychaetes must be mounted on glass slides and identified with a compound microscope. Following identification, samples are labeled and stored for an indefinite time period.

Step 4. Calculate the North Carolina Biotic Index (NCBI) using the following:

$$\text{Biotic Index (BI)} = \frac{\text{Sum}(TV_i)(n_i)}{N}$$

TV_i = ith taxa's tolerance value

n_i = ith taxa's abundance value (1, 3 or 10)

N = sum of all abundance values

The NCBI was derived as another (independent) method of bioclassification to support water quality assessments, (Lenat 1993). This index is similar to the Hilsenhoff Biotic Index (Hilsenhoff, 1987) with tolerance values derived from the NC database. Biotic indices are based on a 0-10 scale, where 0 represents the best water quality and 10 represents the worst.

The Biotic Index for a sample is a summary measure of the tolerance values of organisms found in the sample, relative to their abundance.

BI and BIEPT may not measure impacts that are largely due to sediment, especially if measurements are conducted after a period of scour when sediment-tolerant species ("stable-sand" community) have not yet been established, or chironomids are sparse. In this instance, there may be a change in habitat quality,

but no change in water quality. Similar communities will be found both above and below the source of sediment, but abundances will be sharply reduced in the sediment-impacted area. Both taxa richness and abundance values will be lower at impacted sites. For sites where such habitat changes are the primary cause of stress, the biotic index rating should be used with caution and discussion of results should clearly note the influence of sediment and flow. Tolerance values used as listed in NCDENR Biological Assessment Unit SOP (NCDENR, 2003).

Step 5. 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, other taxonomists in the Biological Surveys Unit verify identifications. Taxonomic assistance is obtained from specialists when appropriate**.**

Background

Macroinvertebrates are useful biological monitors because they are found in all aquatic environments, are less mobile than many other groups of organisms, and are of a size which makes them easily collectable. Moreover, chemical and physical analysis for a complex mixture

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of pollutants is generally not feasible. However, aquatic biota exhibit 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).

Benthos surveys may be requested whenever there is a need for information to support a NCDOT project. Benthic surveys are utilized when there is on site mitigation involving streams. The success of a restoration project can be monitored by investigating the benthic community (what species are found at a site), before and after the stream work is done. Benthos are also used to monitor any effects that road construction may have on streams in the project area. These surveys may be conducted throughout the state. There are no benthic macroinvertebrates listed by the USFWS as endangered or threatened, but there are several crayfish and dragonflies that are listed as Federal Species of Concern.

Policy, Regulatory, and Legal Requirements

- None

Warnings and Precautions

- None

Resources and Tools

- USGS Gage Stations Information
- Coastal Streams Habitat Form
- Mountain Streams Habitat Form
- NCDWQ Biological Assessment Unit
- NCDWQ Protocols

Contacts

- For suggestions to change this procedure contact: Karen Capps (919) 431-2003
- For questions about performing this procedure contact: Logan Williams (919) 431-6617 or Kathy Herring (919) 431-6641

User Access

- Intended for NCDOT Internal Use Only, but not exempt from the public records access requirements

Flowchart

- None

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