New Hampshire Department of Environmental Services (NHDES) Protocols for Macroinvertebrate Collection, Identification and Enumeration

A1 Title and Signature Page

Document Title:	NHDES Protocols for Macroinvertebrate Collection, Identification and Enumeration
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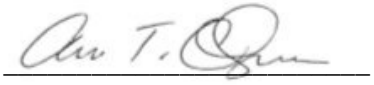
Preparer's Signature:	
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A2 Table of Contents

A3 Introduction (History, Justification)

The primary goals of biological assessment programs are to determine "aquatic life use" status for applicable waterbodies, make decisions for specific permitting and regulatory actions, assist in setting planning and management priorities for waterbodies in need of controls, and prepare water quality reports.

Since 1997, NH DES has collected biological data from wadable streams with the goal of developing indices that can be used to estimate the overall ecological integrity of the biological community. Current New Hampshire water quality standards (Env-Wq 1700) define Biological and Aquatic Community Integrity as the ability to "maintain a balanced, integrated, and adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of similar natural habitats of a region". The indices developed under the bioassessment program are meant to provide a numeric interpretation of the narrative standard outlined above.

A4 Definitions

Macroinvertebrate: An organism without a backbone that is large enough to be seen without a microscope.

River Left: To determine river left, face downstream. River left is on your left hand side.

River Right: To determine river right, face downstream. River right is on your right hand side.

Rock Basket: A device used to collect macroinvertebrate samples from streams. Rock baskets are placed in the streams and left there for six to eight weeks, allowing macroinvertebrates to colonize on the rocks. Detailed description below.

Sieve Bucket: A bucket with a bottom made of very fine wire mesh. It is used to drain water and separate out the debris and macroinvertebrate samples.

A5 Field Procedures

A5.1 Equipment and Materials

Table 1.

NECESSARY MATERIALS PER SITE		
Deployment	**Collection**	
• 3 rock baskets • 1 piece of rebar • Flagging tape • Flow meter • Plastic zip-ties • Sledge hammer • Camera • Site Data Sheet	• 3+ wide mouth jars (1 liter) • 6 labels • large wrench (removal of rebar) • plastic cable ties • garden clippers • 3 sieve buckets	• 3 five gallon buckets • small scrubbing brush per person • flow meter • at least 2 bottles of ethyl alcohol

A5.2 Sample and Data Collection

Rock Basket Deployment
Rock baskets are comprised of regionally indigenous bank run gravel ranging in size from 1.5 - 3.0 inches in diameter (coarse gravel) and contained in a 6.5 inch diameter cylindrical plastic coated wire basket 11 inches in length. The bottom of the basket is hinged and secured with three plastic cable ties. The mesh opening is 1 square inch (Figure 1).

1. Place baskets in riffle habitats or at the base of riffles when possible, at depths that cover the artificial substrate by at least 5 inches (1 - 1.5 feet is generally adequate).
    - Each station uses three replicate baskets that are anchored to the streambed by pounding a ½ inch diameter steel reinforcing rod (e.g. rebar) into the bottom of the stream.
    - The baskets are placed downstream, replicate #1 being placed first on river right in a side-by-side array pattern with a loop of nylon coated steel cable placed over the rebar (see Figure 1). The top-most basket and cable is therefore on river left.

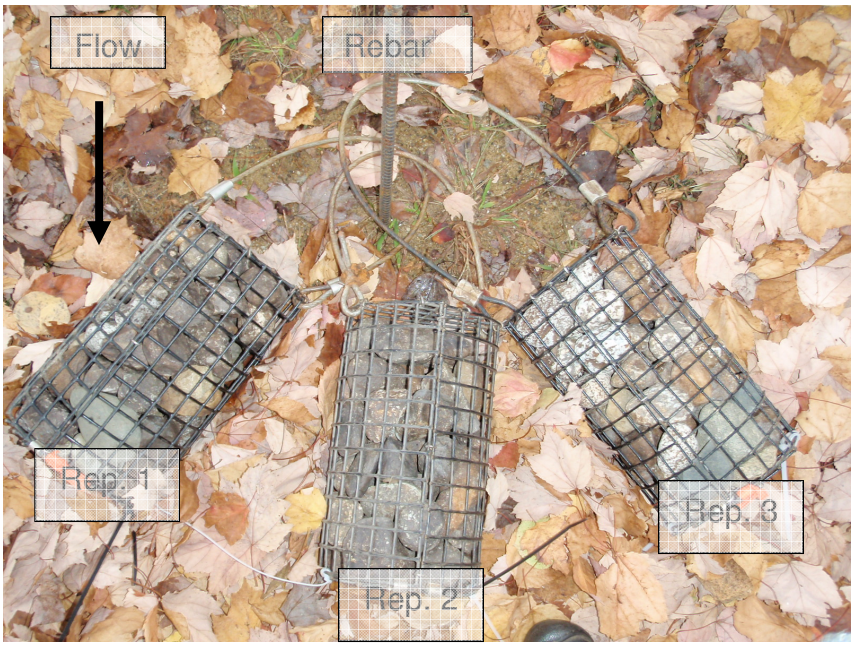

Figure 1. Rock baskets in an array of 3, secured to a "rebar" with nylon coated steel cable.

2. In an effort to deter removal by vandals, all three basket cables are secured to the rebar with a plastic cable-tie.

3. Flagging tape is placed on the rebar at the water level at the time of deployment and on a nearby tree for easy retrieval.

4. Stream velocity and depth measurements are taken at the upstream end of each rock basket and noted on the field data sheet.

5. Deployment of the baskets should occur from July through August. Retrieval occurs from late August through early October.

6. While in-situ, baskets are left undisturbed for six to eight weeks in order for adequate colonization to take place on the substrates. The baskets should be retrieved within a couple days of the allotted time frame.

Rock Basket Retrieval

1. To begin rock basket retrieval, approach the baskets from downstream.

2. Locate and snip the plastic cable-tie that secures the three baskets to the rebar.

3. Place a 3-gallon sieve bucket with a bottom of 600-µm pore size metal screen against the stream bottom with the opening facing upstream just below the target rock basket (Figure 2a).

- Debris and algae clinging to the rock basket should be included in each respective replicate sample.

4. Lift the top basket off the rebar and quickly place it inside the bucket.

5. Lift the sieve bucket out of the water, transport it streamside, and nest it inside a 5-gallon pail that is approximately 2/3 filled with stream water to keep the organisms from drying out.

6. Repeat steps 3-5 for remaining rock baskets.

7. Once all rock baskets have been collected, use garden clippers to cut the plastic tie wraps securing the basket's hinged door and empty the contents of the basket into the sieve bucket. Take note of the replicate number for proper labeling.

8. Place the sieve bucket in the stream at a depth approximately ½ the height of the bucket or nest the sieve bucket inside the 5-gallon pail to keep the organisms from drying out while processing.

9. Carefully inspect the empty wire mesh basket removing all observed organisms by hand and placing them in the 5-gallon pail. Then, using a soft bristle brush, gently scrape the outside and inside of the basket while submerging it in the 5-gallon pail. When done, place the basket upside down for refilling with rocks.


10. Remove rocks one-by-one from the sieve bucket and gently "wash" them using your hands in the 5-gallon pail. Once washed, inspect the rock to ensure that all organisms have been removed. Transfer "clean" rocks to the basket for refilling. In some cases a moderately stiff nylon bristled brush may be helpful in washing rocks, however, be careful not to "over scrub" the rocks as it will compromise the condition of the organisms.

11. After washing each of the rocks, pour all water and material in the 5-gallon bucket into the sieve bucket.
    - Additional stream water can be added carefully to the 5-gallon bucket and poured through the sieve bucket to ensure that all organisms are transferred to the sieve bucket.
    - Visually inspect the inside of the 5-gallon bucket and remove the remaining organisms, placing them in the sieve bucket.
    - At this point all material and associated organisms should be contained in the sieve bucket.


12. Being careful not to spill any of the sieve bucket's contents, partially submerge the sieve bucket in the stream with the bottom facing upstream and use the stream current to wash the material to one side of the sieve bucket.

13. Transfer all material from the sieve bucket into a 1-liter wide mouth jar.
    - Holding the jar inside the sieve bucket during this process will help to minimize the loss of material.

- Repeated washings as described in step 6 are usually necessary to make sure all the material from the sieve bucket gets transferred to the sample container.
- Finally, inspect the sieve bucket to be sure there are no organisms remaining.

14. The sample container is uniquely identified by 2 labels; 1 placed inside and 1 taped to the outside. Be sure to use pencil and include the sample identification number, stream name, replicate number, date of rock basket deployment, date of rock basket collection, method of collection (rock baskets), and entity that completed the sampling (NH DES). See attachment A for example label.


15. Fill the sample container with enough 70% ethanol to cover all the material plus 2 - 3 cm for sample preservation.
    - In some cases more than one jar may be necessary to contain all the material from each rock basket.
    - Do not fill any sample container more that 3/4 full of material.
    - If additional containers are needed, be sure to indicate this on the labels using a note such as "1 of 2 containers, 2 of 2 containers." This will help facilitate laboratory processing.

16. Re-secure the hinged door of the filled rock basket with three plastic cable ties.

17. Repeat steps 7-16 for each replicate. Be sure that each replicate is preserved in a unique sample container.

A6 Laboratory Procedures

A6.1 Sample Processing - Laboratory

See attachment B for the current contractor's Standard Operating and Quality Assurance Quality Control Procedures for processing of macroinvertebrate samples.

A7 Quality Assurance and Quality Control-Field

Three rock baskets are collected which provides us with three replicate samples. Data is examined after analysis to identify any significant discrepancies between these replicates.

A8 Quality Assurance and Quality Control-Laboratory

The contract laboratory performs quality control checks for macroinvertebrate identification and enumeration by submitting 10% of samples to a third party laboratory. Submitted samples are evaluated for three types of errors as shown in the following table.

Error Type	Description
I	**straight disagreements**
II	**hierarchical differences**
III	**missing specimens**

This information is then evaluated for 1) precision of the total number of individuals and 2) precision of total number of taxa and 3) taxa similarity. The first two are a measure of precision while the last is a measure of accuracy. All precision and accuracy quality control measurements must be within 10%. When greater than 10%, the NHDES Biomonitoring Program consults with the contract laboratory to verify failure of quality control. If verified, data is flagged that QC failed and the data is not used for assessment purposes.

A9 Data Sheets

See attachment C for the data sheet used for macroinvertebrate collection. The datasheet is used for multiple purposes, one of these being for recording rock basket deployment and retrieval for macroinvertebrate sampling.
See attachment D for the data sheet used for macroinvertebrate identification and recording.

Attachment A: Macroinvertebrate Sample Label

River Name =
Site ID =
Rep # =
Deploy Date =
Collect Date =
Collect Method =
Collector = NHDES

Attachment B: Macroinvertebrate Sample Processing, Laboratory

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I. DESCRIPTION OF LABORATORY QUALIFICATIONS AND CERTIFICATIONS

Lotic Inc. (Lotic) is an environmental consulting firm located in Unity, Maine. As specialists in the identification of freshwater macroinvertebrates and the evaluation of benthic communities, they are recognized as one of the leading firms in the field of macroinvertebrate taxonomy and ecology. Because of this expertise, they are used by various state and federal agencies. Lotic is currently a contracted taxonomy laboratory for the Maine Department of Environmental Protection and the New Hampshire Department of Environmental Services. Lotic has also worked for the Connecticut Department of Environmental Protection, the Massachusetts Department of Environmental Protection and the Department of Defense. Lotic has conducted macroinvertebrate identifications for the USGS in samples from Pennsylvania, Maine and Wisconsin. Within the private sector, Lotic's clients include power companies, paper mills and other industrial facilities. Lotic biologists are trained in the identification of all major groups of benthic macroinvertebrates, including the Chironomidae and Oligochaeta, and are experienced in a wide range of collection techniques. In addition to benthic macroinvertebrate taxonomy, Lotic has provided Whole Effluent Toxicity (WET) testing for municipal and industrial wastewater treatment facilities for over ten years.

John Tipping, NABS certified taxonomist (level 2, groups 2 and 3) and Senior Entomologist at Lotic, will serve as the project lead and sole point-of-contact. The identification work will be performed by Mr. Tipping with rigorous internal QA/QC to ensure the highest level of taxonomic data quality. Lotic has the demonstrated capacity to process over 500 samples a year in a timely and accurate manner.

Laboratory Procedures

Sample Receipt

Samples will be checked against the chain of custody and logged in the sample log book upon receipt. All samples will also be checked for proper preservation and re-preserved in 70% ethanol if found to be less than adequately preserved. Any containers received leaking or broken

will be replaced, and also noted in the sample log book. A copy of the chain of custody will be retained by Lotic.

Subsampling and Sorting

Samples will be poured through a 500 micron sieve and rinsed with water to remove as much sediment as possible. Large debris, such as rocks and leaves, are washed to remove clinging organisms. The sample or subsample is sorted under a stereo microscope.

Lotic has the capability to perform subsampling using gridded trays, Caton samplers, Fulsom splitters, and the Maine DEP volumetric funnel. When subsampling is required, the unsorted material is retained and preserved for future reference.

Organism Identification

Macroinvertebrates will be identified using state-of-the-art stereo microscopes and the most recently published taxonomic references. Chironomidae are cleared by immersion in a 10% solution of room-temperature KOH for 24-48 hours. Once cleared the specimens are slide mounted in a CMC-10. Once the mountant has dried, the coverslips are ringed with clear nail polish. Oligochaeta are mounted directly in CMC-10. All slide material is identified with a compound microscope. Slides are labeled with the site location, date and sample number.

Organisms will be identified to genus/species unless the condition of the organism or lack of workable keys prevents it. All identified organisms will be retained in the original sample containers, with the exception of the voucher collection.

Sample Disposal

Sample remnants will be retained by Lotic for 5 years or until notified by the client, at which time they will be safely disposed of.

Quality Assurance/Quality Control

Internal Taxonomic Quality Assurance

All sample specimens will be identified to genus or species as allowable by specimen condition and maturity. 10% of the samples identified by each taxonomist will be set aside for re-identification by the other project taxonomist If taxonomic agreement (as determined with the Bray-Curtis Index of Similarity) is less than 95%, the taxonomists will discuss the differences, identify where errors were made, and take corrective action.

As a routine component of Lotic QA/QC protocols, a voucher collection is assembled of at least three specimens (when possible) of every taxon identified for each project. This collection will be retained by Lotic until requested by the client or permanently archived to resolve any taxonomic issues. Each vial in the voucher collection will be labeled with the taxon name, sample ID, sample date, taxonomist, and any other relevant sample information.

External Taxonomic Quality Control

Lotic and its associates maintain professional contacts with numerous research taxonomists and systematists for taxonomic verification of unusual or rare specimens. Any uncertain unusual taxa will be sent to one or more of these outside experts for verification. All samples subject to QA/QC procedures, along with the results of those procedures, will be recorded in the QA/QC log book.

Data Entry and Reporting Quality Assurance

After data entry in Microsoft Excel format, a qualified taxonomist will check 25% of the completed data sheets against the original bench sheets. If no errors are found, the check is complete. If any errors are found, then all data sheets are checked against the database and all errors are rectified. Any necessary corrections will be noted in the QA/QC log book.

Attachment C: Macroinvertebrate Data Sheet

RIVER MONITORING SITE DATA SHEET (Page 1 of 2)

SITE ID: _____ **TOWN:** _____

STREAM NAME: _____

DIRECTIONS: _____

_____

SITE DESCRIPTION: _____

_____

LAT: _____ LONG: _____ DA (SQ MI): _____ ELEV(FT): _____ TYPE: WW TW CW

VISITATION

DATE: _____ ACTIVITIES: _____ PERSONNEL: _____

DATE: _____ ACTIVITIES: _____ PERSONNEL: _____

DATE: _____ ACTIVITIES: _____ PERSONNEL: _____

DATE: _____ ACTIVITIES: _____ PERSONNEL: _____

DATE: _____ ACTIVITIES: _____ PERSONNEL: _____

DATE: _____ ACTIVITIES: _____ PERSONNEL: _____

Water Quality Parameters Collected

Date	TP	NO2/NO3	TKN	NH3	Hard	Alk	TSS	E. coli	Al	TOC	Ca	Mg	Na	K	SO4				

FIELD MEASUREMENTS

DATE	TEMP (c)	SP. CONDUCTANCE (µmhos)	pH	DO (% Sat)	DO (mg/L)	meter

RIVER MONITORING SITE DATA SHEET (Page 2 of 2)

STATEWIDE HABITAT FORM COMPLETED: Y / N DATE COMPLETED: _____

ALGAE SAMPLING COMPLETED: Y / N

ABUNDANCE SURVEY Y / N: Date(s) _____; _____; _____

COMPOSITION SAMPLE Y / N: Date(s) _____; _____; _____

INVERTEBRATE SAMPLE INFORMATION

DATE OF ROCK BASKET DEPLOYMENT: _____ DATE COLLECTED: _____

DEPLOYMENT DEPTH (FT):: _____ RB CONDITIONS @ COLLECTION: _____

STREAM VELOCITY (FT/S):: #1 _____; #2 _____; #3 _____ (#1 IS RIVER RT.)

FISH SAMPLE INFORMATION

SAMPLED Y / N; DATE SAMPLED: _____; PERSONNEL: _____(T);_____

WETTED WIDTH (M):_____; REACH LENGTH (M)_____; SHOCK UNIT:_____

SETTINGS:_____(v); _____(hz);_____(dc); SHOCK TIME (Sec.):_____

VISIT OBSERVATIONS

DATE:_____; Notes:_____

_____

DATE:_____; Notes:_____

_____

DATE:_____; Notes:_____

_____

DATE:_____; Notes:_____

_____

DATA LOGGER DEPLOYMENT INFORMATION

METER/SONDE::_____

DATE DEPLOYED : _____; PERSONNEL :_____

DATE COLLECTED: _____: PERSONNEL :_____

DEPLOYMENT CONDITIONS:_____

_____

GRADIENT ELEVATION CHANGE (FT):_____; LENGTH OF REACH (M/FT):_____

Attachment D: Macroinvertebrate Data Sheet for Identification and Recording.

NHDES		**Sample ID:**	02E-NSR
Sample Date:	9/21/2010	**Water Body:**	N. branch Sugar River
Replicate:	1	**% Subsample:**	50
# of Grids:	18		

Taxa	**Count**	**Stage**	**Excluded**	**Comments**
Acroneuria abnormis	1	l		
Capniidae	1	l		immature
Brachycentrus	1	l		
Hydroptila	2	l		
Cheumatopsyche	5	l		
Ceratopsyche	12	l		
Maccaffertium	68	l		
Epeorus	14	l		
Leucrocuta	1	l		
Ephemerellidae	2	l	X	immature
Isonychia	1	l		
Plauditus	1	l		
Baetis	1	l		
Tricorythodes	1	l		
Ephemerella	2	l		
Eurylophella	1	l		
Chironominae	3	l		
Orthocladiinae	1	l		
Hemerodromia	1	l		