STATE OF TENNESSEE

DEPARTMENT OF
ENVIRONMENT AND CONSERVATION

Division of Water Pollution Control

Quality System
Standard Operating Procedure

for

MACROINVERTEBRATE
STREAM SURVEYS

2011

This SOP is an intra-departmental document intended to govern the internal management of the Tennessee Department of Environment and Conservation and to meet requirements of the U.S. Environmental Protection Agency for a quality system. It is not intended to affect rights, privileges, or procedures available to the public.

DIVISION OF WATER POLLUTION CONTROL
QUALITY SYSTEMS STANDARD OPERATING PROCEDURES
FOR MACROINVERTEBRATE STREAM SURVEYS

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DIVISION OF WATER POLLUTION CONTROL

QUALITY SYSTEM STANDARD OPERATING PROCEDURE FOR MACROINVERTEBRATE STREAM SURVEYS

TITLE AND APPROVAL PAGE

DOCUMENT TITLE	Quality System Standard Operating Procedure for Macroinvertebrate Stream Surveys
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PLAN COVERAGE	General instructions for macroinvertebrate stream surveys in Tennessee

APPROVALS AND CONCURRENCES

Approvals. This is to certify that we have reviewed this document and approve its contents.

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TDEC-BOE Quality Assurance Manager

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Division of Water Pollution Control
QSSOP for Macroinvertebrate Stream Surveys
Revision 2
Effective Date: March 2002
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REVISIONS AND ANNUAL REVIEW PROCEDURE: QS-SOP FOR MACROINVERTEBRATE STREAM SURVEYS

1. This document shall be reviewed annually to reconfirm the suitability and effectiveness of the program components described in this document.

2. A report of the evaluation of effectiveness of this document shall be developed at the time of review and submitted to appropriate stakeholders. Peer Reviews shall be conducted, if necessary and appropriate. It shall be reconfirmed that the document is suitable and effective. It shall include, if necessary, clarification of roles and responsibilities, response to problem areas and acknowledgement of successes. Progress toward meeting TDEC–BOE mission, program goals and objectives shall be documented. Plans shall be made for the upcoming cycle and communicated to appropriate stakeholders.

3. The record identified as “Revisions” shall be used to document all changes.

4. A copy of any document revisions made during the year shall be sent to all appropriate stakeholders. A report shall be made to the Assistant Commissioner and Quality Assurance Manager of any changes that occur. Other stakeholders shall be notified, as appropriate and documented on the “Document Distribution” list.

NOTICE OF REVISION(S) RECORD 2006

Date	Specific Section or Page	Revision Type (major or minor)	Revision Description
10-1-03	xii	Minor	Replace MEFO recipient
10-01-03	II/B/1	Minor	Clarify Station Naming Protocol
10-01-03	II/D/4	Minor	Additional Information for Habitat Assessments
10-01-03	II/D/5 Table 1	Major	Revised Regional Habitat Guidelines
10-01-03	II/F/5	Major	Provide Biorecon Scoring Guidelines for 73a
10-01-03	II/F/6 Table 2	Major	Revised family level biorecon assessment guidelines
10-01-03	II/F/8 Table 3	Major	Revised Genus level biorecon assessment guidelines
10-01-03	II/G/1	Minor	Add online assessment database as source for determining ecoregions.
10-01-03	II/G/2	Minor	Clarify procedures for additional SQKICK sampling to insure 200 organisms sample.
10-01-03	II/G/4	Minor	Clarify procedures for additional modified SQKICK sampling to insure 200 organism sample
10-01-03	II/G/5	Minor	Clarify procedures for additional SQBANK sampling to insure 200 organism sample.
10-01-03	Appendix A 2-7	Major	Updated biocriteria tables.
10-01-03	Appendix A 8-14	Major	Added location and status to ecoregion reference stream table. Added new reference streams.
10-01-03	Appendix A 15-16	Major	Added Table of regional expectations for individual habitat parameters.
10-01-03	Appendix B 4-7	Minor	Revised header information on habitat assessment field sheets.
03-03-03	Appendix B 12	Minor	Revised macroinvertebrate assessment report sheet.
10-01-03	Appendix C 2	Major	Added Peltoperlidae to list of intolerant macroinvertebrate families for biorecons.
10-01-03	Appendix C 3-6	Major	Updated intolerant macroinvertebrate genera for biorecons.

Date	Specific Section or Page	Revision Type (major or minor)	Revision Description
10-01-03	Appendix C 7-21	Major	Updated NCBI scores for Tennessee Taxa.
10-01-03	Appendix C 22-25	Major	Added taxa to list of clinger organisms.
10-01-03	Appendix E	Major	Added taxa to verified taxa list.
10-12-06	V	Minor	Change Commissioner's name
10-12-06	V	Minor	Change QA manager's name.
10-12-06	VIII	Minor	Update reviewers
10-12-06	X	Minor	Update notice of revisions.
10-12-06	Section 1.1, Protocol B, Page 2	Minor	Add naming scheme of UT to UT
10-12-06	Section 1.1, Protocol C, Page 1	Minor	Update meter specifications to match chemical QSSOP.
10-12-06	Section 1.1, Protocol F, page 4	Minor	Clarify how chironomids are counted in richness metric for biorecons.
10-12-06	Section 1.1, Protocol F, Page 6 and 7	Major	Tables 2 and 3 updated based on reference data.
10-12-06	Section 1.1, Protocol J Page 2	Minor	Clarification of Slide labeling procedure.
10-12-06	Section II, Protocol K Page 2 Item f	Major	%NUTOL replaced %Dominant
10-12-06	Appendix A	Major	Biocriteria tables updated based on new reference data. Tables separated by season, metric ranges and target scores adjusted. Bioregion 66f combined with 66deg.
10-12-06	Appendix A	Major	Update ecoregion reference stream list.
10-12-06	Appendix B	Minor	Revised header information on habitat assessment field sheets, stream survey field sheet and biorecon field sheet.

Date	Specific Section or Page	Revision Type (major or minor)	Revision Description
10-12-06	Appendix B	Minor	Macroinvertebrate Assessment report revised.
10-12-06	Appendix C	Minor	Added additional taxa to NCBI score list.
10-12-06	Appendix C	Minor	Added Nymphoridae to list of clingers.
10-12-06	Appendix E	Minor	Added taxa to the verified taxa list.

This revision(s) has been reviewed and approved. It becomes effective on: 10 – 23 - 2006

Paul E. Davis 10/24/06

Paul Davis **Date**
Director
Division of Water Pollution Control

Charles L. Head 10-23-06

Charles Head **Date**
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NOTICE OF REVISION(S) RECORD 2011

Date	Specific Section or Page	Revision Type (major or minor)	Revision Description
10-21-10	Section I.D	Minor	Updated Health and Safety Warnings and Cautions.
10-21-10	Section II.A	Minor	Added provision to relocate ecoregion reference sites upstream if localized problem develops.
10-21-10	Section I.C	Minor	Added definitions and acronyms.
10-21-10	Section I.I Protocol .A	Major	Added sample priority list.
10-21-10	Section I.I Protocol A	Major	Added biological sample decision making flowcharts.
10-21-10	Section I.I Protocol A	Minor	Added provision for intermittent discharges to site selection protocol.
10-21-10	Section I.I Protocol A	Minor	Clarified site selection.

Date	Specific Section or Page	Revision Type (major or minor)	Revision Description
10-21-10	Section I.I Protocol B	Minor	Added quick field reference method summary.
10-21-10	Section I.I Protocol B	Minor	Updated station naming protocols.
10-21-10	Section I.I. Protocol D Table 2.	Major	Re-calibrated habitat assessment guideline scores. Removed impairment categories.
10-21-10	Section I.I Protocol D Table 2.	Major	Added habitat assessment guidelines for headwater streams.
10-21-10	Section I.I Protocol D Appendix B	Major	Revised habitat assessment protocols and field sheets.
10-21-10	Section I.I Protocol F	Major	Revised biorecon voucher requirements for family.
10-21-10	Section I.I Protocol F	Minor	Clarified biometric calculation information.
10-21-10	Section I.I Protocol F Tables 3 and 4.	Major	Recalibrated biorecon scoring guidelines.
10-21-10	Section I.I Protocol F Tables 3 and 4.	Major	Added biorecon scoring guidelines for headwater streams.
10-21-10	Section I.I Protocol G	Minor	Added shallow streams to modified kick protocol.
10-21-10	Section I.I Protocol H.	Major	Added supply and bottle acquisition procedure.
10-21-10	Section I.I Protocol H	Minor	Updated logging information.
10-21-10	Section I.I Protocol H	Minor	Added sample transport information to protocol H.
10-21-10	Section I.I. Protocol J	Minor	Clarified report preparation information. Added digital picture submittal.
10-21-10	Section I.I Protocol L	Major	Added protocol for scoring SQSH in streams that do not fit biocriteria guidelines.
10-21-10	Section I.I. Protocol L Appendix A	Major	Calibrated %NUTOL to Tennessee taxa, renamed %TNUTOL.

Date	Specific Section or Page	Revision Type (major or minor)	Revision Description
10-21-10	Section I.I. Protocol L Appendix A	Major	Replace %EPT with %EPT-Cheum.
10-21-10	Section I.I. Protocol L	Minor	Rearranged order of biometrics to correspond with biometrics tables.
10-21-10	Section I.I Protocol L	Major	Removed biological condition table 4. Added information on index interpretation (pass/fail).
10-21-10	Section I.J.	Minor	Update data and records management.
10-21-10	Section I.I Protocol M	Major	Added protocols for reference stream site selection.
10-21-10	Section II	Major	Added corrective actions to QA/QC.
10-21-10	Appendix A	Minor	Updated reference streams table. Added headwater reference stream table.
10-21-10	Appendix B	Minor	Added list of exotic plants.
10-21-10	Appendix B	Minor	Revised macroinvertebrate assessment report.
10-21-10	Appendix C	Major	Updated Appendix C
10-21-10	Appendix D	Minor	Updated taxonomic keys
01-06-11	Appendix C and E	Major	Combined Verified taxa list, NCBI scores and clinger designation into one list called Tennessee taxa list 2011.
02-09-11	Section I.H	Minor	Changed alcohol acquisition procedure.
02-09-11	Section I.I.	Minor	Changed procedure for transporting samples to lab.
5-2-11	Section I.C and I.I Protocol E	Minor	Clarified location of canopy measurements.
5-2-11	Section I.I Protocol A	Minor	Clarified sample priorities.
5-2-11	Section I.I Protocol B	Minor	Clarified stream mile measurements.
5-2-11	Section I.I Protocol B	Major	Revised naming protocols for unnamed tribs.

Date	Specific Section or Page	Revision Type (major or minor)	Revision Description
5-2-11	Section I.I Protocol D	Major	Added further clarification for scoring of habitat assessments especially for channel flow status and channel alteration categories.
5-2-11	Section I.I Protocol F	Minor	Added clarification in biorecon method.
5-2-11	Section I.I Protocol F Table 4 and 5	Major	Recalibrated scoring criteria for family and genus level biorecons.
5-2-11	Section I.I Protocol G	Minor	Added clarification in SQSH method.
5-2-11	Section II	Major	Added clarification for biorecon vouchers and reference collections.
5-2-11	Appendix A	Major	Revised biocriteria tables (drainage area, taxonomic level, recalibrated ranges). Added headwater tables.
5-2-11	Appendix B	Major	Revised stream survey field sheet.
5-2-11	Appendix B	Major	Revised Habitat assessments field sheets to further clarify category scoring.
5-2-11	Appendix B	Major	Revised biorecon field sheet
5-2-11	Appendix E	Major	Added criteria for taxonomic experts adapted from NABs.
5-16-11	Section I.I Protocol J	Minor	Clarification of tolerance value sources for North Carolina Biotic Index.
5-25-11	Appendix D	Minor	Added supplemental taxonomic keys Updated list of taxonomic specialists

This revision has been reviewed and approved. It becomes effective on July 1, 2011.

Paul E. Davis /by GPW 6/9/11

Paul Davis, Director
Division of Water Pollution Control

Date

Charles L. Head 6/9/11

Charles Head
TDEC Quality Assurance Manager

Date

EVALUATION PROCEDURE:
QS-SOP FOR MACROINVERTEBRATE STREAM SURVEYS

As this document is used, needed changes or improvements will be apparent. Specific recommendations for improvements or changes are solicited as well as information concerning typographical or formatting errors.

1. Copy this page and complete all questions. Electronic versions of this are encouraged especially if comments are significant.

2. Send specific recommendations for improvements or changes, along with the following information, to:

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Document Effective Date	______________________
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Comments	______________________
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QS-SOP DOCUMENT DISTRIBUTION LIST

Copies of this document were distributed to the following individuals in TDEC and TDH

Additional copies were distributed to non-TDEC agencies and individuals upon request (including other state and federal agencies, consultants, universities etc.). An updated distribution list is maintained in the Planning and Standards Section.

The system for document distribution is described in TDEC-BOE Quality Manual, Chapters 5 and 10.

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PREFACE

The U.S. EPA requires that a centrally planned, directed and coordinated quality assurance and quality control program be applied to efforts supported by them through grants, contracts or other formalized agreements. This includes the implementation of a Quality Management Plan as written by the contract holder with Data Quality Objectives (DQOs) set in Quality Assurance Project Plans (QAPPs) for specific projects. The organization may elect to support portions of the QAPP through technical or administrative standard operating procedures (SOPs), as specified by the quality system. As a contract holder and through memoranda of agreement, the Tennessee Department of Environment and Conservation is required to maintain such a system.

This quality system technical Standard Operating Procedure (QS-SOP) was prepared, reviewed and distributed in accordance with TDEC's Quality Management Plan and other quality system documents in response to U.S. EPA's requirements for a Quality Management Program. QS-SOPs are integral parts of successful quality systems as they provide staff with the information to perform a job properly and facilitate consistency in the quality and integrity of the process.

This QS-SOP is specific to the Division of Water Pollution Control and is intended to assist the division in maintaining their quality control and quality assurance processes and ensure compliance with government regulations. It provides specific operational direction for the division's Quality Assurance Project Plan for Macroinvertebrate Stream Surveys.

I. PROCEDURES

I.A SCOPE, APPLICABILITY AND REGULATORY REQUIREMENTS

The purpose of this Quality Systems Standard Operating Procedure (QS-SOP) is to support the Quality Assurance Program. The document provides a consolidated reference document for use in training and orientation of employees. This guide will also be a reference tool for more experienced employees. It establishes an approach that can be recommended to sister agencies that monitor Tennessee water or stipulated to members of the regulated community given monitoring requirements in receiving streams. This SOP describes the macroinvertebrate stream survey process and will delineate all steps in the process, including habitat assessments, field collections, sample analysis, data reduction and reporting. This SOP is only intended to describe routine conditions encountered during a macroinvertebrate stream survey.

Federal Statutory Authority

Federal Water Pollution Control Act (amended through P.L. 106-308, October 13,2000) as Amended by the Clean Water Act of 1977 enacted by Public Law 92-500, October 18, 1972, 86 Stat. 816; 33 U.S.C. 1251 et. seq.
Title III, Sec. 302: Water Quality Related Effluent Limitations
Title III, Sec. 303: Water Quality Standards and Implementation Plans
Title III, Sec. 304: Information and Guidelines
Title III, Sec. 305: Water Quality Inventory

Tennessee Statutory Authority

Tennessee Water Quality Control Act of 1977 (Acts 1971, ch. 164, § 1; 1977 ch. 366, § 1; T.C.A., § 69-3-101 et seq.

Tennessee Regulatory Authority

General Water Quality Criteria and the Antidegradation Statement: Rule 1200-4-3
(specifically 1200-4-3-.03(3) j: Biological Integrity and 1200-4-3-.06 Tennessee Antidegradation Statement)
Use Classification for Surface Waters: Rule 1200-4-4

I.B METHOD SUMMARY

This document describes procedures for performing two types of macroinvertebrate surveys approved by the Division of Water Pollution Control for assessing biological integrity of streams. The entire procedure is described including protocols for sample collection, habitat assessment, sample analysis, data reduction and reporting.

Macroinvertebrates are used by the Division as indicator organisms to determine if a stream supports fish and aquatic life. Two types of surveys (biorecons and semi-quantitative single habitat) are used depending on the purpose of the survey.

Biorecons (BR) will be used as a screening or reconnaissance tool to provide a quick evaluation of the relative health of the biological community. The biorecon will be used primarily for general watershed assessments and for determining where more intensive monitoring is needed. This method is not comparable to biocriteria referenced in the Water Quality Standards.

Semi-quantitative single habitat surveys (SQKICK or SQBANK) will be conducted whenever a more defensible and/or definable assessment is needed. This method is directly comparable to biocriteria referenced in the Water Quality Standards. The semi-quantitative biological survey is also preferred in situations where the use attainment status of a stream is not obvious from the results of a biorecon. Tier evaluations under the Antidegradation Policy, enforcement actions and TMDL studies are additional examples of occasions when biorecons may provide inadequate amounts of information and a semi-quantitative sample would be preferable. It is recommended that this method be used by any outside agency or private organization submitting biological data to the Division for review. The semi-quantitative method is required for any individual conducting macroinvertebrate surveys for permit compliance.

Habitat assessments (high gradient and low gradient) are also described in this document. Habitat assessments are to be conducted in conjunction with all types of biological surveys since habitat is often a limiting factor to the complexity of the benthic community. By following this assessment procedure, habitat can either be confirmed or eliminated as a cause of stress to the macroinvertebrate community.

Macroinvertebrate Survey Quick Field Reference
(Minimum tasks to be completed at all biological sampling sites)

1. Upon arrival at site, record lat/long in decimal degrees, verify that sample location is correct.

2. Establish minimum 100 meter reach area (walk bank without disturbing stream).

3. Take field measurements near middle of reach area (minimum DO, temp, pH and conductivity). Record on stream survey field sheet.

4. Collect chemicals (if needed) upstream of area disturbed during field measurements.

5. Collect macroinvertebrate sample (biorecon or SQSH).

6. If biorecon collected and score is ambiguous, collect SQSH if assessment cannot be made based on field observations (see flow chart).

7. Collect periphyton samples if required.

8. Measure flow (only if chemicals were also collected).

9. Measure canopy midstream in middle of riffle where macroinvertebrates are collected or in middle of stream reach if collecting SQBANK or multiple riffles. Estimate average of entire reach. If periphyton are collected at same time, measure midstream at 5 transects instead.

10. Complete stream survey field sheet including stream sketch.

11. Complete habitat assessment field sheet.

12. Take pictures of upstream/downstream sample reach, any habitat problems and potential pollution sources.

I.C DEFINITIONS AND ACRONYMS

Benthic Community: Animals living on the bottom of the stream.

Biocriteria: Numerical values or narrative expressions that describe the reference biological condition of aquatic communities inhabiting waters of a given designated aquatic life use. Biocriteria are benchmarks for water resources evaluation and management decisions.

Biometric: A calculated value representing some aspect of the biological population's structure, function or other measurable characteristic that changes in a predictable way with increased human influence.

Bioregion: An ecological subregion, or group of ecological subregions, with similar aquatic macroinvertebrate communities that have been grouped for assessment purposes.

Ecoregion: A relatively homogenous area defined by similarity of climate, landform, soil, potential natural vegetation, hydrology, and other ecologically relevant variables. There are eight (Level III) ecoregions in Tennessee.

Ecological Subregion (or subecoregion): A smaller area that has been delineated within an ecoregion that has even more homogenous characteristics than does the original ecoregion. There are 25 (Level IV) ecological subregions in Tennessee.

Ecoregion Reference: Least impacted waters within an ecoregion that have been monitored to establish a baseline to which alterations of other waters can be compared.

Habitat: The instream and riparian features that influence the structure and function of the aquatic community in a stream.

Headwater stream: Streams with less than a two square mile drainage area. Typically first or second order.

Macroinvertebrate: Animals without backbones that are large enough to be seen by the unaided eye and which can be retained by a U.S. Standard No. 30 sieve (28 meshes/inch, 0.595 mm).

Reference database: Biological and chemical data from ecoregion reference sites.

Riparian Zone: An area that borders a waterbody (approximately 18 meters wide).

Stream Order: Strahler order as determined using 7.5 series topographic maps.

Watershed: The area that drains to a particular body of water or common point.

Acronyms

BR	Biorecon
BOE	Bureau of Environment
Cfs	Cubic feet per second
CO	Central Office
COC	Chain of Custody
DO	Dissolved Oxygen
D/S	Downstream
ECO	Ecoregion Reference Stream
EFO	Environmental Field Office
EPT	Ephemeroptera Plecoptera Trichoptera
EPT-Cheum	EPT abundance excluding *Cheumatopsyche* spp.
ES	Environmental Specialist
FECO	Headwater Reference Stream
GIS	GeographicInformation System
GPS	Global Positioning System
HW	Headwater
IT	Intolerant Taxa
LDB	Left Descending Bank
MSDS	Material Safety Data Sheets
NCBI	North Carolina Biotic Index
OC	Oligochaeta and Chironomidae
PAS	Planning and Standards Section
PFD	Personnel Flotation Device
QA/QC	Quality Assurance/Quality Control
QAPP	Quality Assurance Project Plan
QSSOP	Quality System Standard Operating Procedure
RDB	Right Descending Bank
RM	River Mile
SQBANK	Semi-Quantitative Bank Sample
SQKICK	Semi-Quantitative Kick Sample
SQSH	Semi-Quantitative Single Habitat Sample
TDEC	Tennessee Department of Environment and Conservation
TDH	Tennessee Department of Health
TMI	Tennessee Macroinvertebrate Index
TMDL	Total Maximum Daily Loading
TNUTOL	Tennessee Nutrient Tolerant Organisms
TOPO	Topographic Map
TR	Taxa Richness
U/S	Upstream
WPC	Water Pollution Control

I.D HEALTH AND SAFETY WARNINGS

(Adopted from Klemm et al., 1990)

1. Know how to swim and/or use a PFD when entering the water.

2. Always wear waders with a belt to prevent them from filling with water in case of a fall. In high velocity and high flow streams it is advisable to wear a PFD.

3. Follow Tennessee boating laws and regulations. Information is available through the Tennessee Wildlife Resources Agency. PFD are required when operating a boat.

4. Be vigilant, especially in turbid streams, to avoid broken glass, beaver traps or other hazardous objects that may lie out of sight on the stream bottom. Heavy wading boots should be worn in these situations.

5. Keep first aid supplies in the office, lab and field at all times. Training in basic first aid and cardio-pulmonary resuscitation is strongly recommended.

6. Any person allergic to bee stings or other insect bites should carry needed medications and instruct team mates on how to use in the event of an allergic reaction.

7. Always perform lab work involving ethanol or CMCP-10 in a room containing a properly installed and operating hood.

8. Carry communication equipment in the field in case of emergency.

9. Keep a file in the office that contains emergency contacts and physician's name for each employee.

10. Consider all surface waters potential health hazards due to toxic substances or pathogens and minimize exposure as much as possible. Do not eat, drink, smoke, apply cosmetics or handle contact lenses while collecting samples. Avoid splashing face and clean exposed body parts (face, hands and arms) immediately after contact with these waters. Carry soap and an adequate supply of clean water, disinfectant wipes and/or waterless sanitizer for this purpose.

11. If working in water known or suspected to contain human wastes, get immunized against tetanus, hepatitis, typhoid fever and polio.

12. Try to avoid working alone in the field. When working alone, make sure the supervisor or their designee knows where you are and when you are expected to return. Check in periodically.

13. Material Safety Data Sheets (MSDS) sheets for ethanol and CMCP-10 (if mounting slides) are to be kept in the lab or office. Everyone working with these agents should be familiar with the location and content of the MSDS sheets.

14. Be aware of potentially volatile situations. If possible, obtain permission from land-owners before crossing private property. Have business cards available to leave at residences when appropriate. If approached by someone representing law enforcement, show them your state I.D. and ask to see their I.D. or badge. The Tennessee Highway Patrol can be reached by dialing *THP (*847) from your mobile phone.

15. When traveling in a vehicle always wear a seat belt and follow all Tennessee Department of Safety and Motor Vehicle Management rules.

16. In the event of a life threatening emergency, go the nearest hospital. Call for emergency assistance if moving the injured person is likely to inflict further injury. If a non-life threatening injury occurs on the job, seek medical assistance from the authorized state worker's compensation network. A current list of providers may be found on the State Treasurer's homepage under Workers Compensation Provider Directory at www.tn.gov/treasury. Always complete and file an accident report if medical assistance is provided for a work related injury

I.E CAUTIONS

1. Avoid cross contamination of samples. Thoroughly rinse all nets and sieves and inspect for clinging organisms before leaving the sample site. Inspect nets and sieves again immediately before sampling the next site. Use new sample bottles whenever possible, otherwise thoroughly rinse bottles and inspect before use.

2. Avoid sampling bias by following these procedures exactly. Document any deviation.

3. Take care not to over-sample, especially on biorecons. Take care not to undersample (less than 160 organisms) on SQSH samples.

4. Make sure sample site meets ecoregion, drainage area and sample method requirements before comparing to semi-quantitative or biorecon guidelines. Biocriteria metrics used to calculate the TMI can only be applied to SQKICK or SQBANK samples. Never calculate quantitative metrics or apply biocriteria to biorecons.

5. Use the standardized station ID naming protocol for all samples. Check water quality database to make sure a station has not already been established with a different station ID. Notify PAS of any discrepancies. Make sure the station ID is included on all paperwork associated with the sample.

6. Measure stream length from mouth to headwaters. When measuring embayments, measure length of original channel from confluence with the original channel of the main stem. Use GIS (preferred) or map wheel at the 1:2400 (7.5 minute) scale to measure stream miles. When using GIS use the ArcView measuring tool, do not use the Reach File Index or the NHD flowline layer which measures in straight lines. Do not use TDEC on-line assessment map measuring tool as it is inaccurate due to rounding errors. The USGS site http://water.usgs.gov/osw/streamstats/tennessee.html may be used.

7. Use 7.5 minute (1:2400) USGS topographic maps or GIS mapping layers to determine Strahler stream order (do not use Gazetteer).

8. To avoid errors, calibrate all meters at the beginning of each day unless overnight travel is required. The meters should minimally be calibrated once a week. Perform a drift check at the end of each day (or on return if overnight travel is required). If the meter calibration is off by more than 0.2 units for pH, temperature or D.O. when measured in mg/L or by more than 10% for conductivity or D.O when measured in % saturation, precede all measurements between the initial calibration and the drift check with an N (questionable data) on the stream survey field sheet and on any Chemical Request Forms (if it is likely that measurements are erroneous record IF for instrument failure). Indicate reason for not recording measurement or for questionable measurements on the stream survey field sheet.

9. If sample request forms have already been submitted, notify the Planning and Standards Section of questionable readings in writing (e-mail or fax).

10. Express all time on a 24-hour (military) clock format.

11. Write all dates in mm/dd/yy or mm/dd/yyyy format.

12. Express all distance measurements in meters, except flow. Flow is expressed in cfs.

13. Make sure to use appropriate units for all field measurements as indicated on the stream survey field sheet.

14. Use GPS to confirm location at site. Record latitude and longitude in decimal degrees.

15. If an error is made in any documentation, draw a single line through the error, so that it is readable and write the correction above. Date and initial the correction.

16. When possible, chemical and semiquantitative (SQSH) macroinvertebrate samples should be collected on the same day (required for CADDIS analysis). If this is not possible, chemical and biological samples should not be separated by more than 4 weeks.

17. Make sure drainage area, sample method and ecoregion drainage are appropriate for comparison to biorecon guidelines or SQSH biocriteria.

18. Check water quality database stations table before assigning station names to make sure a name has not already been assigned to the site by another sampling team or agency. Check station Ids to make verify names follow logical progression from downstream to upstream.

19. Use caution when assessing headwater streams (< 2 square mile drainage) with biorecon guidelines and/or SQSH biocriteria tables. Guidelines are based on a limited number of samples within each bioregion.

20. Take care that additional stream information recorded on the stream survey field sheet does not contradict information provided on the habitat field sheet. This is especially important for sediment and riparian information.

I.F INTERFERENCES

1. Document all deviations from protocol.

2. Semi-quantitative bank samples collected in 65j, 66d, 66e, 66f, 66g, 67f, 67g, 67h, 67i, 68a, 68b, 68c, 69d, 71e, 71f, 71g, 71h, and 74a cannot be compared to biocriteria. If sampling in a non-riffle stream in these regions, an upstream or offsite reference must be collected.

3. Semi-quantitative kick samples collected in 65a, 65b, 65e, 65i, 73a and 74b cannot be compared to biocriteria. An upstream or offsite reference must be collected.

4. Additional samples (of the same habitat) should be collected if needed to ensure 200 organisms were found in the standard collection (document).

5. Avoid sampling in flooded conditions or immediately after a flood.

6. Do not sample if stream is reduced to isolated pools. If stream channel naturally goes dry, only sample if there has been flow for longer than 30 days. Only compare to biocriteria or biorecon guidelines for those regions where reference streams routinely went dry (68b, 68c, 69d, and 71i).

7. Flag dissolved oxygen, pH, temperature and conductivity readings with an N (Questionable data) if post-trip drift checks show meter calibrations to be off by more than 0.2 units (or 10% for conductivity).

8. Organisms that are marked verified on the Tennessee taxa list (Appendix C) must be sent to the state lab for verification and inclusion in the statewide reference collection. After in-house confirmation, the state lab will send any new taxon to a qualified expert for verification (Appendix D).

9. Sampling stations should be located in areas where the benthic community is not influenced by atypical conditions, such as those created by bridges or dams, unless judging the effects of atypical conditions is a component of the study objectives.

I.G PERSONNEL QUALIFICATIONS

Tennessee Civil Service Titles: Biologist or Environmental Specialist (state employees only). For the purpose of this report, both position titles will be referred to as biologist.

Minimum Education Requirements: B.S. in a biological science. Coursework in stream ecology and macroinvertebrate taxonomy is desirable. A graduate level degree in stream ecology, aquatic biology or similar field is preferable.

Minimum experience: one year (specific class-work involving biological stream surveys and macroinvertebrate taxonomy can be substituted for experience)

Expertise: Macroinvertebrate taxonomy (must be able to consistently pass quality control checks), computation of basic statistics, use of standard water quality monitoring meters, habitat evaluations and general water quality assessments.

Training: Protocols outlined in this SOP
Quality System Requirements
Quality Assurance Project Plan for 106 monitoring

I.H. EQUIPMENT AND SUPPLIES

Prior to any sampling trip, gather and inspect all necessary gear. Replace or repair any damaged equipment. Calibrate all meters the morning of the sampling trip with a drift check at the end of the day (or the end of the trip for overnight stays). Upon return from a trip, take care of any equipment repairs or replacements immediately. Necessary equipment will vary per project, but the following is a standardized list.

Field Equipment

Waders
Forceps
Ethanol
External sample tags
Internal sample tags
Habitat Assessment Field Sheet (High gradient for riffles, Low gradient for glide-pool)
Stream Survey Field Sheet
Biorecon Field Sheet (Biorecons only)
Biological Analysis Request Form (for Chain of Custody and/or samples sent to lab)
Topographic maps (USGS quadrangle maps) may also be referred to as topos or quads.
Tennessee Atlas and Gazetteer
½ gallon wide mouth plastic sample bottles for Semi-Quantitative samples
Small wide mouth plastic bottles for biorecons
Calibrated GPS unit
Calibrated Dissolved Oxygen meter and replacement membrane kit
Calibrated pH meter
Calibrated conductivity meter
Calibrated temperature meter or thermometer in ^{o}C
Spare batteries for all electronic equipment
Camera (preferably digital) with memory cards or film
Triangular dip net with 500-micron mesh (Biorecons and SQBANK samples only)
One meter square kick net with 500 micron mesh (SQKICK samples only)
Rectangular net (18”) with 500 micron mesh (SQKICK in small streams only)
Sieve bucket with 500 micron mesh
White enamel or plastic pans for sorting debris (biorecons only)
Magnifying lens
Waterproof marking pens (Sharpies), pencils and black ballpoint ink pens (not roller-ball)
Flashlights
Duct Tape
First Aid Kit
Time keeping device
Spherical densiometer (for canopy measurements)
Map Wheel or GIS capability (to calculate stream miles to assign station ID in field if needed)
Cell phone desirable (or other method for contacting help in emergency)

Laboratory Equipment

The following equipment is needed to perform sample analysis (semi-quantitative samples only).

Dissecting Microscope
Compound Microscope
Jewelers Forceps
Petri dish
Microscope slides
Round 12 mm coverslips
Square 22 mm coverslips
Gridded Tray with subsampling insert
Small Gridded dish (36 grids)
Ethanol
CMCP-10 or equivalent permanent mounting media
Random number jar
Taxonomic Bench Sheet
Turkey baster (or equivalent suction device)
Transfer pipette (or equivalent suction device)
Slide storage box
Glass vials with rubber or Teflon line lid (for reference specimens)

The following equipment is needed for laboratory confirmation of biorecons

Dissecting Microscope
Jewelers Forceps
Petri dish
Ethanol
Glass vials with rubber or Teflon line lid for reference specimens

Sample container and Ethanol Acquisition

Sample containers and ethanol are to be obtained through the Tennessee Department of Health Environmental Laboratory in Nashville.

Supplies must be requested at least two weeks prior to the anticipated date they will be needed (preferably one month

Item	Old Inventory No.	Edison Number
SQSH Jar 1/2 gal	200-08210	1000109388
1 oz wm bottle (Biorecon collection)	200-0190	1000109082
Alcohol Ethyl	400-0700	1000109487

Contact: Dr Bob Read, Director, TDH Environmental Lab
(615) 262-6302
Bob.read@tn.gov

I.I PROCEDURES

Protocol A - Selection of Survey Type and Station Location

Biologist or Environmental Specialist from EFO
Central Office Coordinator

1. Determine biological sampling needs.

The central office will coordinate biological sampling needs with the environmental field offices. The location and type of scheduled biological assessments will be included in the annual water quality monitoring workplan. Additional biological assessments may be conducted as needed.

Biological sampling will generally follow the watershed cycle. When developing the monitoring workplan within the targeted watershed, macroinvertebrate samples should be collected with the following priority:

a. Ecoregion reference sites (ECO and FECO).

b. Sites on the 303(d) list for Fish and Aquatic Life

c. TMDL or other sites where nutrients samples are going to be collected (ideally macroinvertebrates should be collected on same day as chemicals at least once to maximize versatility and statistical power of data). It should be noted that nutrient impairment cannot be evaluated in the absence of a macroinvertebrate sample unless other biological evidence, such as excessive algal growth is documented.

d. Sites below point source discharges in wadeable streams **when in-stream biological surveys are not required in discharge or stormwater permits**. Location of point source discharges and permit requirements can be accessed through Waterlog. To download a spreadsheet by watershed go to the WPC reports, permits by watershed cycle, interactive format. Biological surveys submitted by permitted discharges are available in SQSH WPC new and other agency table of Water Quality Database provided to field offices monthly.

e. Sites below ARAP activities in wadeable streams that have been completed since the last watershed cycle where biological impairment is suspected. Emphasis should be placed unpermitted activities, violations and those that are large scale or where there are a dense concentration of smaller alterations. Waterlog can be accessed to get information on ARAP activities. To download a spreadsheet by watershed go to the WPC reports, permits by watershed cycle, interactive format. Examples of ARAP activities where downstream monitoring may be warranted include commercial dredging, stream relocations, impoundments, concentrated or large scale water withdrawals and major road construction.

f. Stream reaches suspected of non-point source (for example large scale development, cluster of stormwater permits, increase of more than 10% impervious surface or other potential problems.

g. Sites where the last sample was ambiguous (collect SQSH at these).

h. Previously assessed segments. (Note that a single site per assessed segment is adequate if assessment was supporting and no changes are evident.)

i. Unassessed reaches (fish and aquatic life) especially in third order or larger streams or in headwaters that have human disturbance and downstream reaches are not impaired.

2. The type of macroinvertebrate sample will be determined based on the type of assessment needed. In general biorecons will be used for routine watershed assessments and screening while SQSH will be used when a more defensible assessment is needed and for clarification of ambiguous biorecons. It can also be used to confirm biorecons where score does not, in the opinion of the biologist, reflect true stream conditions. (For example richness is high but abundance appears low). Figures 1-4 provide guidelines for determining what type of biological sample is most appropriate.

   If chemical and SQSH samples are to be collected from the same site, it is desirable that at least one chemical sample be collected on the same date to provide maximum statistical power and make data useful for other purposes such as criteria development, biometric calibration and correlation analysis. If it is not possible to collect the same day, chemical and SQSH samples should be collected as close as possible (for example the same week or at least the same month). It is required for samples to be collected the same day when conducting CADDIS analysis.

   a. Ecoregion reference sites (ECO and FECO) – Biorecon and SQSH (Figure 1).

   b. NPDES permit compliance, enforcement, nutrient TMDL, Pre/post BMP, Pre/post ARAP, potential ETW, CADDIS – SQSH (Figure 2).

   c. 303(d) list – SQSH or Biorecon (Figure 3).

   d. Watershed Assessment – SQSH or Biorecon (Figure 4).

   It is recommended that biorecons not be used in ecoregion 73 since the intolerant richness metric is not useable.

Biological Sample Decision Making Flowcharts

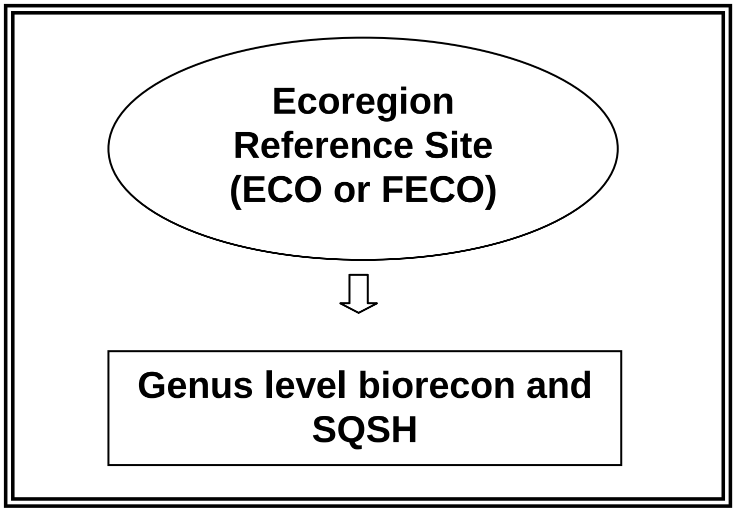


Figure 1: Biological sample decision making chart for ecoregion reference sites.

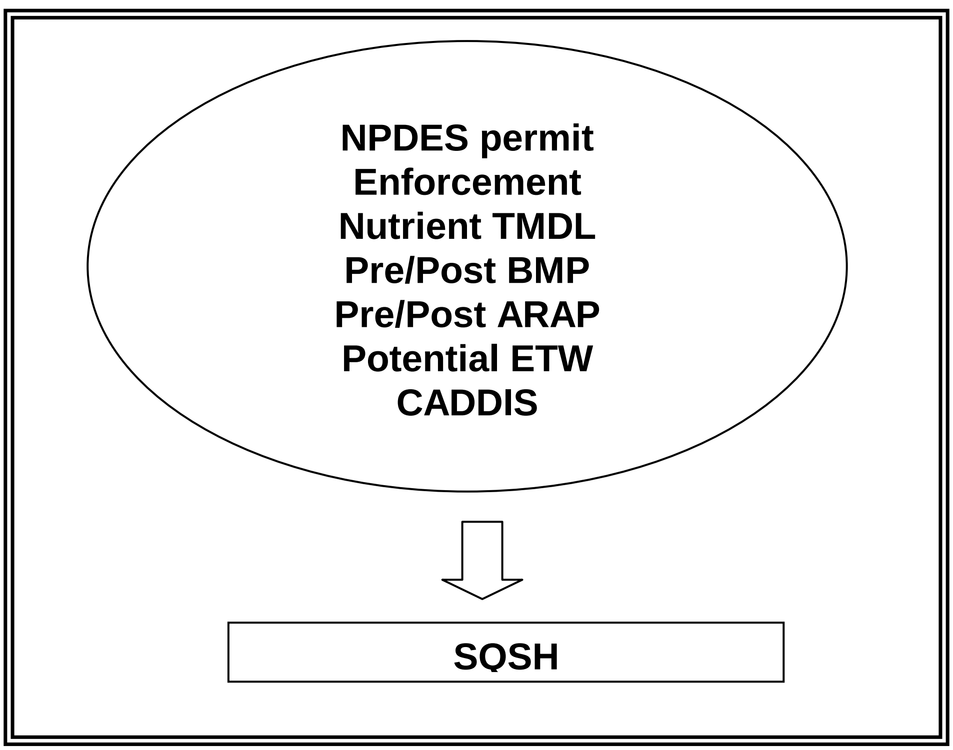


Figure 2: Biological sample decision making chart for NPDES, enforcement, nutrient TMDL, BMP and potential ETW sites.

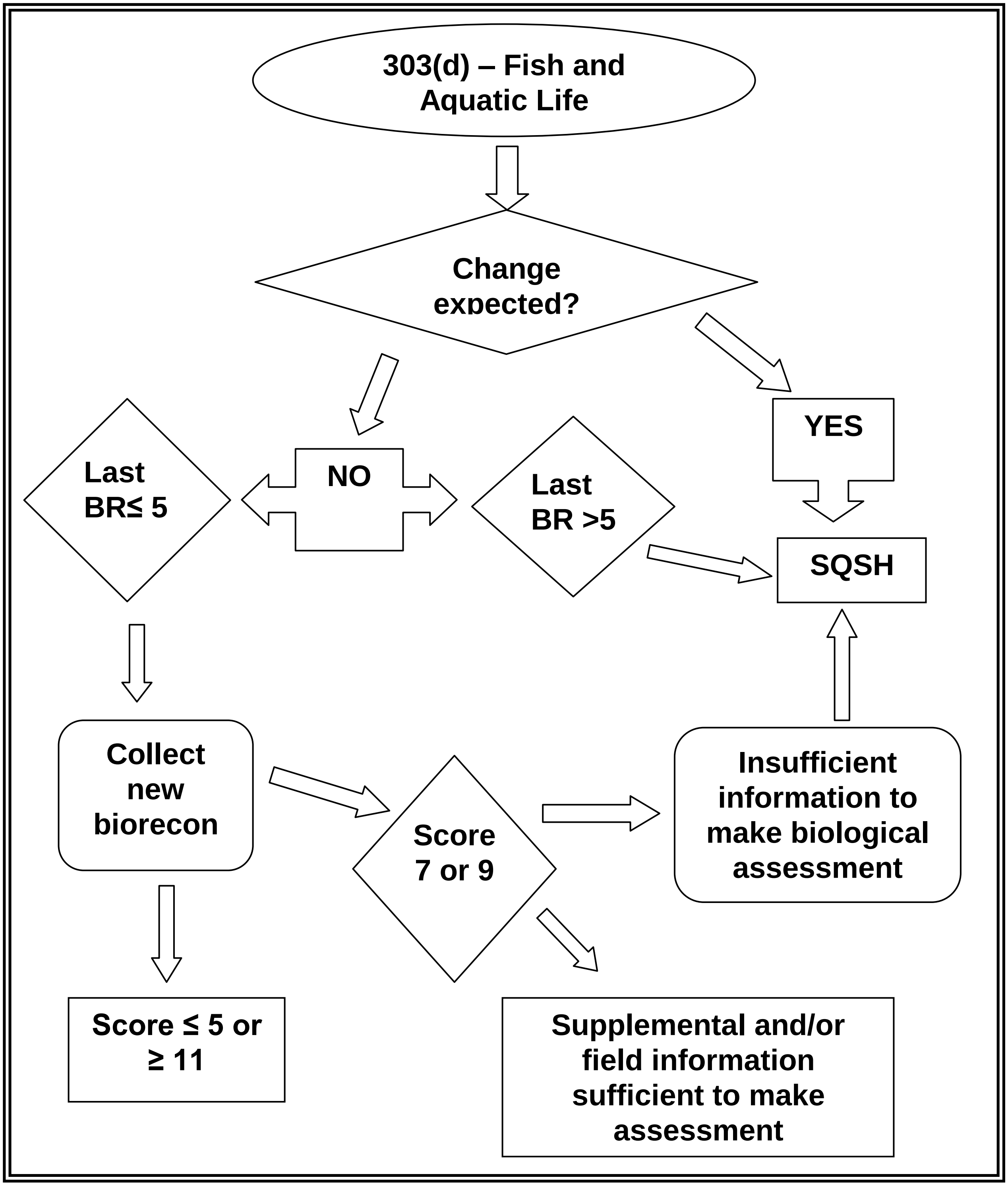


Figure 3: Biological sample decision making chart for 303(d) listed sites.

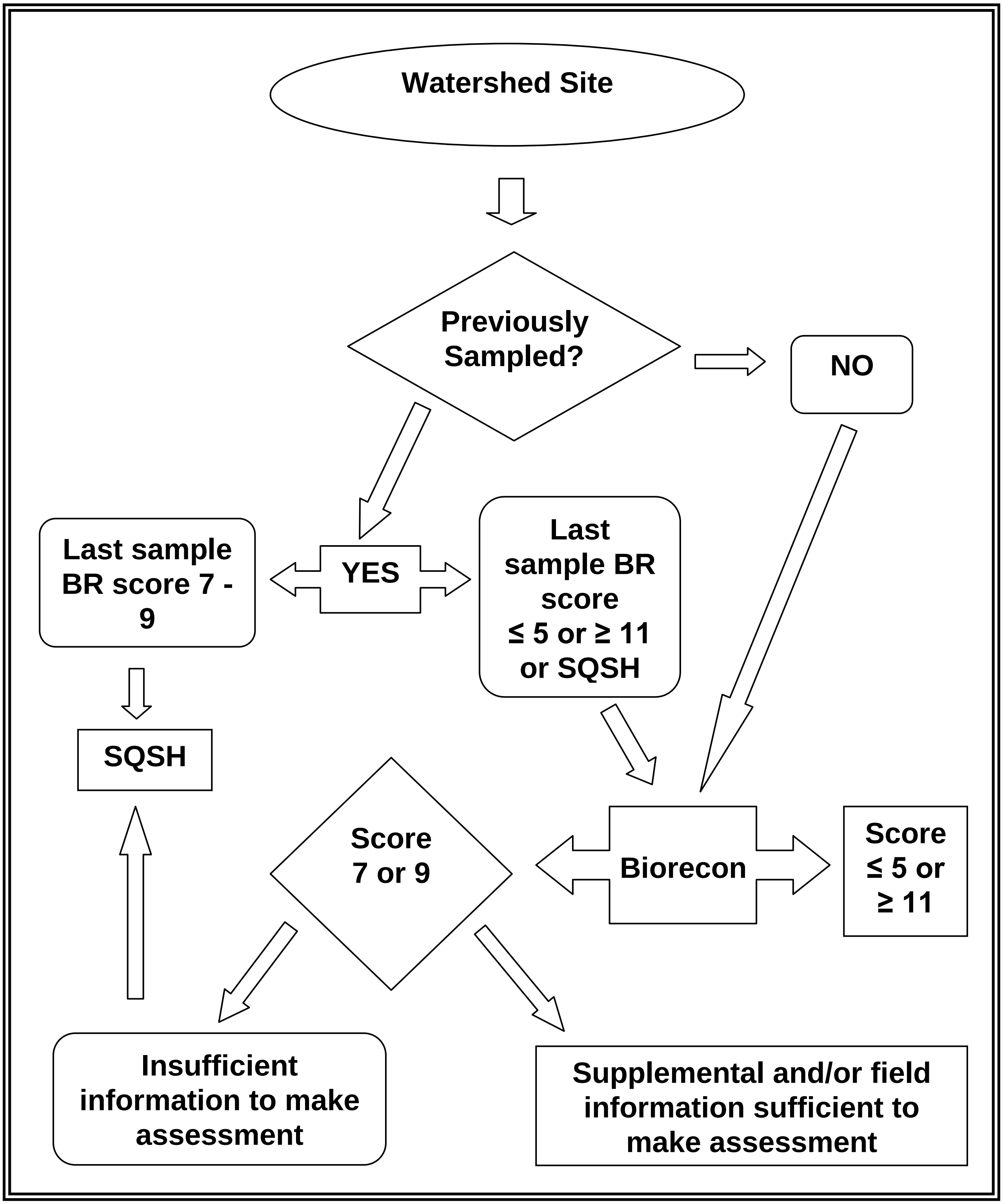


Figure 4: Biological sample decision making chart for watershed sites.

3. Select sites.

Site selection is dependent on the study objectives. After determining the specific objectives of the study and clearly defining what information is needed, select sampling sites on specific reaches of the stream. Reconnaissance of the waterway is very important. Note possible sources of pollution, access points, substrate types, habitat, flow characteristics, and other physical characteristics that will need to be considered in selecting the sampling sites. Although the number and location of sampling stations will vary with each individual study, the following basic rules should be applied:

a. Determine whether an upstream or watershed **reference site** is needed or if the study site can be compared to biocriteria (Appendix A) or biorecon guidelines (Tables 4 and 5) derived from the ecoregion reference database. In order to compare to biocriteria, the watershed upstream of the test site must:

    1. Be at least 80% within the specified bioregion
    2. Be of the appropriate upstream drainage area
    3. Be collected using the collection method designated for that bioregion (SQKICK or SQBANK).

    This information can be found in Appendix A. Compare all appropriate semi-quantitative samples to biocriteria. Depending on study purposes or if the study stream does not meet requirements for the reference database, an upstream sample or an appropriate watershed reference may need to be collected. Instructions for comparing data to an alternate reference are provided in Protocol K.

    Compare biorecons on streams whose upstream drainage is at least 80% within a bioregion to guidelines developed from the ecoregion reference database (Tables 4 and 5). If the test stream crosses multiple bioregions upstream of the test site, select an appropriate upstream or watershed reference. (An alternative is to compare the site to guidelines for each appropriate bioregion, however if assessments differ another reference must be used). Instructions for comparing data to an alternate reference are provided in Protocol F.

b. For watershed screenings, locate sites near the mouth of each tributary. If impairment is observed, locate additional sites upstream of the impaired stream reach to try to define how far the impairment extends and locate potential sources.

c. For monitoring point source pollution, establish a station downstream of the source of pollution in the stream after mixing has occurred. If complete mixing of the discharge does not occur immediately, left bank, mid-channel and right bank stations may need to be established to determine the extent of possible impact. Establish stations at various distances downstream from the discharge. Space the collecting stations exponentially farther apart going downstream from the pollution source to determine the extent of the recovery zone. For intermittent discharges, sampling should be within thirty days of last discharge. An additional site should be located upstream of the discharge point to help identify influences associated with the discharge.

d. For site specific sampling, locations immediately above, or below the confluence of two streams, or immediately below point/nonpoint source discharges, should be avoided if mixing does not immediately occur. Unless the stream is extremely small or extremely turbulent, an in-flow will usually hug the stream bank with little lateral mixing for some distance. This may result in two very different biological populations and an inaccurate assessment of stream conditions. This can be avoided by sampling after mixing has occurred.

e. All sampling stations under comparison during a study should have similar habitat unless the object of the study is to determine the effects of habitat degradation.

f. Sampling stations for macroinvertebrates should be located within the same reach (200 meters or yards) of where sampling for chemical and physical parameters will be located. If the macroinvertebrates are collected more than 200 meters from the chemical sampling , consider it a separate station and assign it a different station ID number unless there are no tribs, discharges, construction, agriculture, road crossings or other activities that would influence the stream between the chemical and biological sampling points.

g. Sampling stations should be located in areas where the benthic community is not influenced by atypical conditions, such as those created by bridges or dams unless judging the effects of atypical conditions is a component of the study objectives.

h. Ecoregion reference sites may be relocated upstream if localized disturbance is observed during sampling (for example beaver activity, riparian disturbance, 4-wheel activity, dredging etc.)

i. Stream must have had flow for a minimum of 30 days prior to sampling. Avoid habitats that may not have been submerged for 30 days, isolated pools or stagnant water.

Protocol B – Assigning Station ID

Biologist/Environmental Specialist

Assign station IDs to each site using the following protocol. The station ID will be used to identify the sample and must be included on all associated paperwork, results, tags etc. This number is to be used to identify this site every time it is sampled for any parameter (benthic, fish, bacteria, chemical). If new stations are set up that will also have chemical or bacteriological monitoring, send the new station information to Planning and Standards as soon as the station location is finalized and before the lab sends results (Usually 30 days). Minimally station information should include station ID, latitude and longitude (in decimal degrees), HUC, ecoregion, stream order and specific location information (such as road crossing) that can be located on a map. If only biological samples will be collected, complete all header information on the stream survey field sheet and send with biological data packet to lab (SQSH) or PAS (biorecon). If the stream is first or second order, drainage area must be indicated.

It is very important that station Ids are assigned consistently with the same location always assigned the same ID regardless of the sample collection type, purpose, samplers or year.

1. **Before assigning a new station ID, check the "current stations" table in the Water Quality Monitoring database to make sure a number hasn't already been assigned to that site**. Even if the site has not been collected before by the EFO, a station ID may have already been assigned based on other agency data (NPDES instream sampling, ARAP, special projects, TVA etc.). Do not assume that a station does not exist because it has not been collected by the EFO. It is very important that all data from a single location be given the same station ID to facilitate assessments based on all available information. Contact the Planning and Standards section if there is any question or if there are naming errors associated with existing stations.

   Unless the sites are located upstream and downstream of a point source discharge, tributary confluence or some other factor that would affect the stream, stations collected within 200 meters of each other are considered the same site**.** (So, if chemical samples were taken off the bridge and biological samples were collected up to 200 meters upstream, they are still the same station.)

   Chemical and biological stations collected more than 200 meters apart can still be considered the same station if there are no tributaries, discharges, construction, agriculture, road crossing or other activities that would influence the stream between sampling points. **It is very important for biological and chemical samplers to coordinate naming of station locations to avoid confusion.**

2. **The official stream name is the one found on the USGS 7.5 minute topographic map or equivalent GIS layer.** Do not use other sources such as gazetteer, TDOT bridge signs or local names, which may differ.

3. **It is also important that river miles used in the station ID are measured as accurately as possible and correspond to the latitude and longitude for easy comparison between multiple stations on the same waterbody.** Only use GIS (preferred), map wheel or http://water.usgs.gov/osw/streamstats/tennessee.html to measure stream miles. Always use the 1:2400 scale. When using GIS use the ArcView measuring tool, do not use the NHD flowline layer or Reach File Index. Do not use TDEC on-line assessment map measuring tool as it is not accurate due to rounding.

   When measuring river miles for streams that enter an embayment, begin measurement from the confluence with the original channel of the main stem (not from where the stream becomes an embayment). For example, in Figure 5, river mile 0 for Bearden Creek would start at the confluence with the original channel of the Clinch River as marked on the topo within Melton Hill Lake. Follow the original stream channel line if marked on the topo (do not use "poly lines"). If the original stream channel is not marked on the topo, straight lines may be used through the embayment area.

   If there are other stations located on the same stream, make sure the assigned river miles are appropriately upstream or downstream of existing stations. If errors are discovered on existing stations, contact PAS to have the stations reassigned.

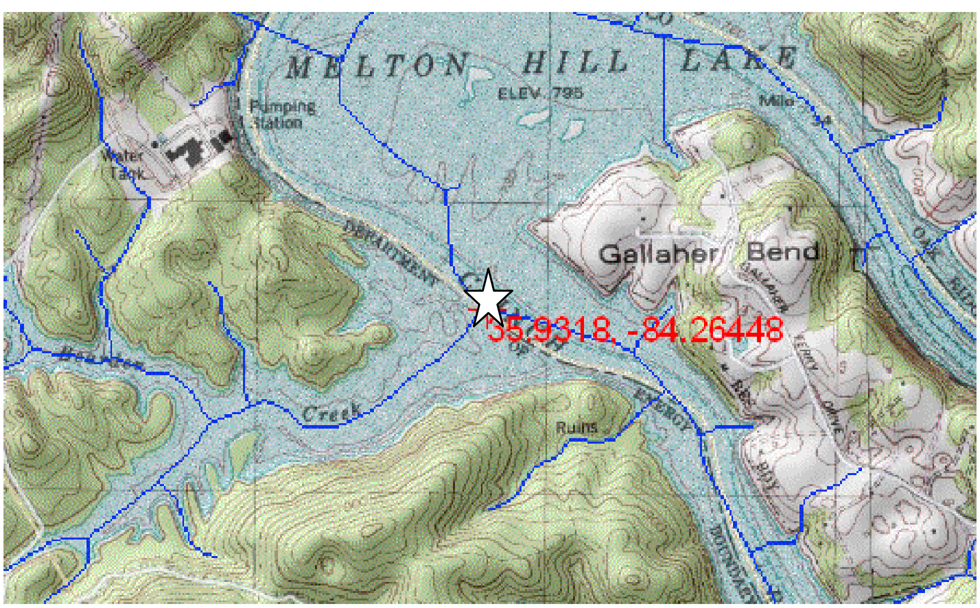


Figure 5: Start of river mile for measuring creeks within embayment areas.

The only exception to the naming scheme is sites that have been designated as Ecoregion or headwater reference sites. These sites are always identified with their ECO or FECO designation no matter what the purpose of sampling. If new ecoregion reference sites are added, contact Planning and Standards (PAS) to determine the appropriate station name.

4. **Named streams/rivers**

If a number does not already exist for the site, create an identification number. All letters in the station name are capitalized.

a. The first five digits will be the first five letters of the stream name (capitalized). If the stream name has more than one word, use the first letter of each word finishing out the five letters with the last word. For example, South Fork Forked Deer River would be SFFDE. Do not use the words River, Creek Branch etc. (Fork is only used if the stream is also designated river, creek, branch etc.) For example, Dry Fork would be DRY but Dry Fork Creek would be DFORK. **The stream name will be one designated on the 24 scale USGS topographical map or GIS layer. (Do not use the Gazetteer, local name, TDOT signs etc.).**

b. The next five characters designate the river mile. This will be written as three whole numbers, a decimal and a tenth space. For example, river mile 1.2 would be written as 001.2. Do not add zeros to make a short stream name longer. It is very important that the river mile be determined as accurately as possible (see number 3 above).

c. The last two characters designate the county (or state if not in Tennessee). Use the County Identification table in Appendix B to determine the appropriate county designation. The county is expressed with two letters; do not use the numeric state code. If the station is in another state, add an underscore __ before the two letter state abbreviation.

Example 1: A station located at river mile 1.5 on Puncheoncamp Creek in Greene County would be PUNCH001.5GE

Example 2: A station located at river mile 25 on the North Fork Forked Deer River in Gibson County would be NFFDE025.0GI.

Example 3: A station that is located in Kentucky at river mile 15.2 of Spring Creek would be SPRIN015.2_KY.

5. Unnamed Streams/Tributaries.

Check a 24k scale topographic map (hardcopy or GIS) layer to see if the unnamed stream is within a named geographical features such as a cove, hollow, gulf, gulch or valley.

a. **For streams that are not within a named geographical feature**:

(1) Use the first five letters of the receiving stream the tributary enters.

(2) Use a 5-character stream mile to indicate where the tributary enters the main stem (whole number, decimal and tenth for example river mile 114.6 would be entered 114.6).

(3) Use the letter T to indicate a tributary.

(4) Use the river mile of the unnamed tributary where the station is located.

(5) Use the two letter county abbreviation from Appendix B. If the station is in another state, add an underscore _ before the two letter state abbreviation.

Example 1: A station located at river mile 0.2 on an unnamed tributary that entered the Harpeth River at river mile 114.6 in Williamson County would be HARPE114.6T0.2WI.

Example 2: A second station at river mile 0.3 on the same trib would be HARPE114.6T0.3WI.

Example 3: A station located at river mile 5.5 on a different unnamed tributary which entered the Harpeth River at mile 115.0 in Williamson County would be HARPE115.0T5.5WI.

(6) When naming an unnamed tributary to an unnamed tributary, start at the named stream (mainstem) and work upstream to the sampling point.

(a) Record the first five letters of the mainstem (named stream).
(b) Record the river mile where the first unnamed tributary enters the main stem followed by a T.
(c) Record the river mile where the second unnamed tributary enters the first one, followed by a T.
(d) Record the river mile where the station is located, followed by the county designation.

Example: A station at river mile 0.5 on an unnamed tributary that flows into a second unnamed tributary at river mile 0.1 which, in turn flows into Turkey Creek at river mile 9.0 in Gibson County would be TURKE9.0T0.1T0.5GI (Figure 6).

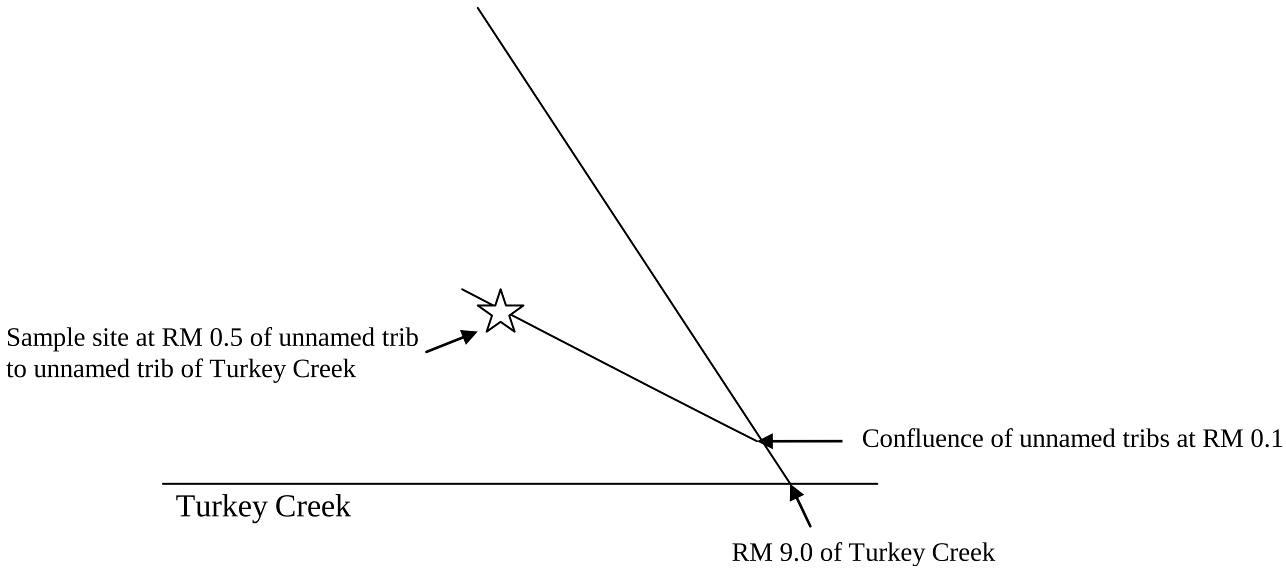


Figure 6: Naming scheme for stations located on unnamed tributaries to unnamed tributaries. Station ID TURKE9.0T0.1T0.5GI

b. **For streams that are within a named geographical feature:**

(1) The first five digits will be the first five letters of the name of the geographical feature (capitalized). If the feature name has more than one word, use the first letter of each word finishing out the five letters with the last word. Do not use the words Cove, Hollow, Gulch, Gulf, or Valley. If the feature name has fewer than five letters use the entire name.

(2) Add the underscore_G to indicate that the station is named after a geographical feature and not a named stream. Streams with "_G" will be the main branch running through the feature.

(3) The next three characters designate the miles upstream from the nearest named stream or waterbody. This will be written as one whole number, a decimal and a tenth space. For example, river mile 1.2 would be written as 1.2. If the stream is an unnamed tributary to the main branch (_G streams), the miles will be measured upstream from the main branch instead of the nearest named stream or waterbody (see example 3).

(4) Use the two letter county or state abbreviation from Appendix A. If the station is in another state, add an underscore _ before the two letter state abbreviation.

Example 1: A station that is in Shingle Mill Hollow in Marion County and is 0.3 miles upstream from Nickajack Reservoir, which is the closest named waterbody would be SMILL_G0.3MI.

Example 2: A station that is located on an unnamed main branch in Cave Cove in Marion County that is 0.4 miles upstream of the nearest named stream would be CAVE_G0.4MI.

Example 3: A station at river mile 0.2 on an unnamed tributary that enters main branch in Cave Cove at river mile 1.0 would be CAVE1.0G0.2MI.

6. Wetlands

a. For named wetlands

(1) Use the first five letters of the wetland name if one word – if more than one word use the first letter of each word plus as many letters are needed in the last word to get five total letters (see 2.a).

(2) Add underscore_W.

(3) Use a 3-character stream mile including one whole number, the decimal and a tenth space. For example river mile 1.2 would be written as 1.2.

(4) Use the two letter county or state abbreviation from Appendix A. If the station is in another state, add an underscore _ before the two letter state abbreviation.

Example 1: A station located at DUCK wetland would be DUCK_W1.2CH.

Example 2: A station located at BLACK HORSE wetland would be BHORS_W1.2CH.

b. For unnamed wetlands with an associated stream

(1) Use the first five letters of the stream associated with the wetland if one word – if more than one word use the first letter of each word up to five letters (see 2. a.).

(2) Add underscore_W

(3) Use a 3-character stream mile including one whole number, the decimal and a tenth space. For example river mile 1.2 would be written as 1.2.

(4) Use the two letter county or state abbreviation from Appendix A. If the station is in another state, add an underscore _ before the two letter state abbreviation.

Example: A wetland associated with a stream Clear Creek would be CLEAR_W1.2SM.

c. For isolated unnamed wetlands with no stream associated with it, use the name associated with the ARAP permit request.

(1) Use the first five letters of the company associated with the wetland, - if more than one word use the first letter of each word up to five letters.

(2) Add underscore_W.

(3) Use a 3-character stream mile including one whole number, the decimal and a tenth space. For example river mile 1.2 would be written 1.2.

(4) Use the two letter county or state abbreviation from Appendix A. If the station is in another state, add an underscore _ before the two letter state abbreviation.

Example: Company name Boones Farm BFARM_W1.2CO

7. Sinking streams (with no clear channel or surface flow to main stem – use standard naming scheme for streams with clear channel or that resurface)

a. Use the first five letters of the stream name if one word – if more than one word use the first letter of each word up to five letters. For unnamed sinking streams or if the receiving stream is unclear use the first five letters of the closest mapped feature.

b. Add underscore _S.

c. Use a 3-character stream mile including one whole number, the decimal and a tenth space (use additional characters as needed if the stream mile is greater than 9.9). Start mileage from the point where the stream disappears (if the stream resurfaces downstream and it is clearly the same stream, estimate the distance between surface points).

d. Use the two letter county or state abbreviation from Appendix A. If the station is in another state, add an underscore _ before the two letter state abbreviation.

Example 1. A station located at river mile 1.2 on Dry Creek would be DRY_S1.2CU.

Example 2. A station located at river mile 11.2 on Stinky Cow Creek would be SCOW_S11.2CU.

Example 3. An unnamed sinking stream station located on Crane Top Ridge with no clear flow pattern would be CTOP_S1.2FR

8. Reservoirs (man-made lakes)

a. Assign the first 5 letters of the impounded stream (or embayment).

b. Use a 5 character stream mile if the sample is collected near the river channel. If the sample is collected near the right or left bank (such as at a boat dock) use a 4 character stream mile and the letter L or R to designate the right or left descending shore.

c. Use the appropriate 2 letter county or state abbreviation from Appendix A. Add an underscore _ before the two letter state abbreviation for stations in another state. For example, a station that was collected from a boat on Fishing Lake which dams Otter Creek in Anderson County would be OTTER012.3AN. If the station was collected off a dock near the left descending shore the station ID would be OTTER12.3LAN.

In the site description include the reservoir name as well as location for clarification (for example Otter Lake near boat dock)

9. Natural Lakes

a. Use the first 5 digits of the lake's name.

b. Using an S to designate station and a two digit whole number, assign the next available station ID. For example if station IDs 1 through 4 already exist on that lake from previous studies (check the water quality database) then use station ID 5. This would be designated S05.

c. Use the appropriate 2 letter county or state abbreviation from Appendix A. Add an underscore _ before the two letter state abbreviation for stations in another state.

For example, a new station located on Reelfoot Lake in Obion County would be REELFS11OB .

Protocol C – Field Parameters

Adapted from U.S. Environmental Protection Agency. 2002

BIOLOGIST/ENVIRONMENTAL SPECIALIST

Dissolved Oxygen, pH, temperature and conductivity measurements are to be recorded at each biological monitoring station every time the site is sampled. Field parameters are to be recorded on the stream survey field sheet (Protocol E). Multi-probe or individual meters meeting the following specifications can be used.

Measure dissolved oxygen, pH, temperature and conductivity before biological samples are collected. (If also collecting chemical or bacteriological samples, measure field parameters after these samples are collected and before flow is measured). Place the probe upstream of where surface water samples were collected. Allow sensors to equilibrate before recording measurements. Document all measurements including duplicates on the stream survey field sheet.

Label all meters as property of the State of Tennessee, Department of Environment and Conservation. Assign each meter a distinct identifying designation, (i.e. letter or a portion of the serial number) for calibration, maintenance, and deployment records. Mark each meter with this designation. Record the meter's ID number on the Stream Survey Field Sheet. Multi-probe or individual meters meeting the following minimum specifications may be used (Table 1). Beyond following the instructions in this SOP for calibrating, maintenance, and logging procedures, it is also recommended to refer to manufacturer's instructions.

Table 1: Water Quality Probe Minimum Specifications

Parameter	Range	Accuracy	Resolution
Temperature	-5 ^{o}C to 45 ^{o}C	+/- 0.20 ^{o}C	0.1 ^{o}C
Specific Conductivity	0 to 100,000*umhos/cm	+/- 1% of reading	4 digits
pH	2 to 12 units	+/- 0.2 units	0.01 units
Dissolved Oxygen	0 to 20 mg/L	+/- 0.2 mg/L	0.01 mg/L

* Areas of mining or other high conductivity/low pH may need a higher range.

1. **Calibrate Meter(s)** – Meters only need to be calibrated if they are going to be used that week. At the beginning of each week or day or within 24 hours of use, calibrate meter(s) for all parameters that will be measured, following the manufacturer's instructions. Conductivity and pH probes are calibrated weekly with a drift check performed daily upon return (or at the end of the sampling period if overnight travel is involved). The drift check can be performed the next morning if time is a factor. The probes must be recalibrated when the drift check is out of the acceptable range, otherwise calibrating these probes once a week is acceptable. A drift check should be performed weekly for temperature. DO probes are to be calibrated each morning of use and at each site where necessary (see # 2). Drift

checks for DO probes are not necessary if the meter was recalibrated in the field. If probes are factory calibrated, check readings against the appropriate standards to ensure the calibration is still accurate. Maintain calibration SOPs for each type and/or brand of meter. Keep all calibration records in a backed up digital format (preferred) or bound logbook (Figure 7). Include the date, meter designation, project name/number, initials of calibrator, parameter, standards used, meter reading, and adjustments. Also, record routine maintenance and repairs in the logbook. Some probes must be sent to the manufacturer for calibration. Other probes must be replaced when they no longer maintain their calibration. In these cases, refer to manufacturer's instructions.

To check the calibration of the temperature probe place an ASTM thermometer in a container of room temperature water large enough to submerge the temperature probe. Place the meter in the water bath and allow it to equilibrate then compare the probe's reading to the thermometer's reading and mathematically adjust the probe's temperature as necessary. Coordinate with TDH laboratory to include the ASTM thermometer in their annual thermometer calibration check against the ASTM certified thermometer. Record this information in the calibration log.

Date	Meter	Project	Init.	Parameter	Standard	Reading	Adj	Comments
3/6/02	YSI-A	Davis Ck	JEB	Conductivity	142	120	142	Cleaned contacts
3/6/02	YSI-A	Davis Ck	JEB	Conductivity	142	140	NA	Drift Check

Figure 7: Meter Calibration Log

2. **Calibrate DO Probe** – The DO probe must be calibrated using either Winkler Titration (mg/l) or air calibration (% saturation) each morning prior to use. Most probes automatically compensate for temperature changes. Some probes also automatically compensate for pressure changes. An ASTM r calibrated thermometer and/or a handheld barometer must be carried in the field if the probe does not compensate for temperature and/or pressure changes. It is only necessary to recalibrate the probe at sites where there is a significant elevation, pressure or temperature change and the meter does not automatically compensate. A significant change in elevation is 1000 feet. A significant change in pressure is ±20 mm Hg (higher or lower) or when a storm front comes through the area. A significant change in temperature includes any ±5ºC change in temperature (higher or lower). If the DO probe is air calibrated, changes in pressure do affect concentration readings. Record the air calibration at the site in a calibration log in the field to the specified resolution in Table 1.

3. **Probe Placement** – Ideally, measure water parameters after collecting chemical and bacteriological samples and before measuring flow or collecting other samples (i.e. macroinvertebrate, periphyton). Turn on the meter(s) and if there is a DO stirrer, be sure it is activated. Carefully place the meter(s) in the thalweg upstream of the chemical and bacteriological sampling area. Suspend the probe(s) in the water column so it does not touch the bottom. If the water is too shallow to suspend the meter(s), carefully lay it on its side on firm substrate (preferably rock). Do not allow the probe(s) to sink into soft substrate.

   Stand downstream of the probe, being careful not to disturb the substrate in the area of the probe(s). Allow enough time for each reading to stabilize before it is recorded. Depending on the meter, it may take a couple of minutes for dissolved oxygen to equilibrate. Record initial readings in the field notebook or the stream survey field sheet to the specified resolution (Table 9).

4. **Duplicate Readings** - Take duplicate measurements at each site. If time is a constraint (short sample holding times or daylight), duplicate readings may be reduced to the first and last site each day. To take a duplicate measurement, lift the probe completely out of the water, wait for the readings to change then return it to the original location or slightly upstream if the sediment was disturbed. Allow the meter to equilibrate before recording readings. If the readings are off by more than 0.2 units for pH, temperature, and DO in mg/L or off by more than 10% for specific conductivity, repeat the procedure until reproducible results are obtained. Record all readings on the stream survey field sheet. All results are to be recorded to the resolution specified in Table 1. Rinse the probes with tap water after use at each site to avoid contamination.

5. **Record Field Parameters** – Document the field measurements on the stream survey field sheet. Specific conductivity must be recorded in umhos/cm or uS/cm, dissolved oxygen in ppm (mg/l), and temperature in ^{o}C.

6. **Drift Check** – Without post-calibration checks, the accuracy of the water parameter measurements cannot be demonstrated. At the EFO lab, perform a drift check on each meter at the end of the day (or at the end of the trip on multiple night trips) and record results in the logbook (Figure 5). Drift checks can be done in the field as long as you have the proper equipment. To check that the probes have maintained their calibration for pH and conductivity, compare the probe's readings against the appropriate pH, and conductivity standards. Adjust calibration if the probe is going to be used again that week. If the meter's calibration is off by more than 0.2 for pH or more than 10% for conductivity, all readings between the initial calibration and the drift check must be marked as questionable (N). To check that the probes have maintained their calibration for temperature, compare the probe's readings against a standard ASTM thermometer. If the meter's calibration is off by more than 0.2, all the readings between the initial calibration and the drift check must be marked as questionable (N). When the DO probe has been air calibrated in the field due to pressure, elevation or temperature changes, a drift check is unnecessary at the end of the day. If the

DO probe was not re-calibrated since leaving the base office, a drift check (Winkler or air calibration) should be performed at the end of the day. If the meter's calibration is off by more than 0.2 mg/L (Winkler) or 10% (air), all readings between the initial calibration and the drift check must be marked as questionable (N). On stream survey field sheets precede all questionable readings with an "N" (questionable data) or line through reading (if measurement is determined to be erroneous) and document reason.

7. **Other Parameters** – some multi-parameter probes contain sensors for other water quality parameters such as turbidity or suspended solids. If these parameters are also measured, they should be calibrated following manufacturer's specifications prior to use with drift checks performed at the end of each week. Duplicate measurements should be taken at each site and recorded on the stream survey sheet.

Protocol D – Habitat Assessment

Biologist/Environmental Specialist

Habitat assessments are primarily used to determine whether various components of the habitat are factors in fish and aquatic life impairment. A qualitative approach is used to minimize field time while still establishing a standardized assessment procedure that can be used for comparison to ecoregional guidelines. Because of the qualitative nature, the habitat assessment is not considered a cause of impairment without a measured biological response. By close adherence to these assessment guidelines and standardized training, a consistent habitat assessment approach can be achieved.

Although there are four categories on the habitat assessment field sheets, the final state assessment is supporting or non-supporting. Likewise, for each parameter, the final state assessment is comparable to reference or not. Therefore, the primary goal of the habitat assessment is for each biologist to score the assessments so that the same conclusion is reached for total habitat and for each parameter at this scale (either supporting or not supporting).

Conduct a habitat assessment every time any macroinvertebrate sample is collected. This assessment must be conducted on the same day the biological sample is collected. Although generally only macroinvertebrate samples are collected, it is important to consider both macroinvertebrates and fish when evaluating habitat. The macroinvertebrate sample is used as an indicator while the habitat assessment is used as a cause of impairment to both fish and aquatic life. It is necessary to walk the entire reach while assessing habitat. It is advisable that two staff members collaborate on the assessment to reduce subjectivity.

Two different habitat assessment field sheets will be used dependent on the Level IV ecoregion and/or stream type at the sampling location (Appendix B). These field sheets are modified from *Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers* (Barbour et. al., 1999). Habitat guidelines for each ecoregion are provided in Table 3. In ecoregions 65j, 66d, 66e, 66f, 66g, 66i, 66j, 66k, 67f, 67g, 67h, 67i, 68a, 68b, 68c, 68d, 69d, 69e, 71e, 71f, 71g, 71h, and 74a as well as moderate gradient streams in 71i, use the High Gradient Stream (formerly Riffle-Run) assessment field sheet to evaluate habitat. In ecoregions 65a, 65b, 65e, 65i, 73a, 73b and 74b as well as low gradient non-riffle steams in 71i, use the Low Gradient (formerly Glide-Pool) assessment field sheet. Low gradient assessments may also be appropriate in some lower reaches of larger streams in other ecoregions. Copies of these field sheets are located in Appendix B. It is recommended that field sheets be copied to waterproof paper for field use.

Ecoregions designation based on sample point lat and long can be found at http://tnmap.tn.gov/wpc/. The Planning and Standards section should be contacted if there is uncertainty about what ecoregion a stream is located in.

Evaluate all ten habitat parameters with 20 being the highest attainable score for each parameter. Scores are divided into four categories (optimal, suboptimal, marginal and poor) with a range of scores possible in each category.

The habitat assessment is based on the entire stream reach (typically 100 meters) with the location of the macroinvertebrate sample the middle of the stream reach. If a longer sampling reach was used to collect macroinvertebrate samples (typically in larger streams or when habitat is scarce) or when collecting fish, the entire sampling reach is used for the habitat assessment. The assessment is an average of the conditions within this reach.

Because this assessment is qualitative, it is essential that assessors follow standardized protocols for scoring especially when assigning categories of Optimal, Sub-optimal, Marginal and Poor. This will enable comparison to ecoregional reference streams that have been assessed following the same standardized procedure.

Two steps are used to assign a habitat score for each parameter. The first step is assigning the parameter a condition category of optimal, suboptimal, marginal, or poor. **Assessors must be careful not to focus on the category names, they are meaningless in the state assessment which is subsequently calibrated to a pass/fail based on the median reference condition for each ecoregion.** The four broad categories are just a convenient tool to quadrisect the various habitat parameters before ranking. These categories are generally based on quantity of the specified parameter. The second step is assigning a numeric score (rank within that category). This is generally based on the quality of the parameter.

It is important that the assessor does not pre-calibrate the site based on reference expectations while in the field (For example thinking that streams in a certain area should score optimal because that is as good as it gets, even if they do not meet the description). Scores will be adjusted to ecoregional reference conditions during analysis (Table 2). The best streams in some ecoregions may never fall in the “optimal” category, but will be considered fully supporting based on comparison to reference condition.

The guidelines on the field sheet and in this document should be followed as closely as possible. If the stream does not fit the descriptions, professional judgment should be used with the comment field used to explain scoring. A comment line is available at the bottom of each parameter. Attach additional pages if needed. The station number, assessor and date should be included on any additional pages. Comments can be used to provide additional descriptions, clarify difficult calls, explain atypical stream conditions, specify what characteristic resulted in the score when there are multiple interpretations, describe any factors that should be taken into consideration when interpreting the score or any other information that would help explain the assessment to a reviewer who has not been to the site.

Header

Header information should be filled out completely It is important to also complete header information on the second page (back) since documents are often sent as pdf files and pages may become separated.

Station ID: Check current stations table in water quality database to see if a station ID has already been assigned to this location. (Do not rely on memory or assume no-one else has ever collected any type of sample at this location. TVA or a permit holder may already have established a station at this location). If a station has not already been assigned, use standard station naming procedure, protocol B. When assigning new stations, make sure the river miles are in line with existing stations (for example river mile 1 should be upstream of river mile 0.5) or notify PAS if existing stations are named inappropriately.

Stream name: Name should match USGS (for example topographic map or GIS coverage), or use unnamed tributary to named receiving stream. Do not use local names that are not on the map.

Station Location: If it is an existing station, use location in current stations table of the water quality database. If it is a new station, use road names or features identifiable on topographic map if possible. Unacceptable location descriptions include:

Upstream STP (specify what STP).

Stinky STP – Specify upstream or downstream and which outfall if there is more than one.

Behind Mr. Jones House. – Mr. Jones may move or next sampler may not know where Mr. Jones lives, use 123 Penny Lane Instead.

Playground (camp site, church, landfill, park etc.) – Use name of playground and road location or other map feature.

Off Highway 123 – Roads are long, add another landmark (for example off Hwy 123 approx. 0.5 mile upstream of intersection with Bumpass Rd.

Highway 123 Bridge – Specify upstream or downstream and how far.

WBID/HUC number: Specify WBID segment (from assessment database) or HUC number if segment number is unknown.

Group: Specify watershed assessment group. This is especially important in watersheds that are split between groups such as 06010102, 06010201 and 06020001.

Habitat Assessed By: Include initials of staff member(s) who scored the habitat assessment. Do not include any team members who were not involved with habitat assessment.

Date: Enter date of assessment (month/day/year)

Time: Enter time (24 hour clock)

Ecoregion: Specify ecoregion of stream reach. If questionable, check station location at http://tnmap.tn.gov/wpc/.

Circle if this is a consensus or duplicate assessment, otherwise leave blank.

Associated Log #: Record the log number of the biological sample collected at the same time. (If both macroinvertebrate and periphyton samples were collected, record the macroinvertebrate log number.)

Fill in Station ID, date and initials of assessor on back of field sheet. (If field sheet is faxed or single-side copied at a later date, this will help identify the second half of the assessment if they are spearated).

Note: If this is a QC, two separate habitat field sheets should be completed independently. Consensus scores may be marked on one of the field sheets (differentiate between investigator and consensus) or a separate sheet may be used.

Protocol D-1: Moderate to High Gradient Habitat Assessment Field Sheet.

The moderate to high gradient habitat assessment field sheet (Appendix B) will be suitable for most wadeable streams in middle Tennessee (except for some streams in the Inner Nashville Basin – 71i) and in east Tennessee. It will also be used for two ecoregions in west Tennessee; the Transition Hills (65j) and Bluff Hills (74a).

If riffles are not present due to disturbances such as sedimentation, sludge deposits or channel alterations, but the slope is moderate to high gradient, these field sheets will still be used to evaluate the stream. Some moderate to high gradient streams naturally do not have riffles (steep mountain streams or moderate gradient bedrock streams) however they should still be evaluated with this field sheet. The only time a low gradient field sheet should be used in these ecoregions is if the stream is in a low gradient area (sometimes occurs near the mouth of large streams). Note that the ecoregion reference guidelines cannot be used in low gradient streams in these ecoregions or for non-wadeable streams. Therefore, a suitable upstream or watershed reference must be selected for comparison. In these cases, the test stream should score within 75% of the "reference" stream to have comparable habitat.

1. Epifaunal Substrate/Available Cover

When assessing this parameter, look at various types of natural structures available to macroinvertebrates and/or fish throughout the entire reach. Look for habitat that provides refugia, feeding, spawning or nursery functions. Do not count "newly fallen trees, leaf litter that is not decaying or unstable habitats that will be washed out. Also do not include artificial habitat such as fish attractors, tires, appliances, rip-rap, etc.

Natural habitats that are typically found in moderate to high gradient streams include:

Cobble riffles
Gravel riffles
Bedrock crevices
Boulders (Fish cover)
Pool rock
Run Rock
Submerged trees (not newfall)
Snags
Decaying leaf litter
Rock overhangs (Fish cover)
Undercut banks
Submerged Roots
Macrophyte beds
Mossy rocks

To assign a condition category, first look at how much of the stream reach is covered by natural, stable, productive habitat. The numeric score (rank) within the condition category is assigned based on the variety and quality of habitat.

For example, in a very high gradient mountain stream, over 70% of the substrate may be available for colonization putting this in the optimal category. Four or more habitats may be present, but is dominated by boulder cover so it may only score a 16. Variations in habitat that provide niches for different faunal types should be considered as different habitat types. For example, cobble in flowing water and cobble in pools count as two types of habitat.

Habitat that is not of sufficient quantity to support faunal populations, does not show evidence of colonization (such as newly fallen leaves), is not productive (such as seamless bedrock) or is likely to wash out should not be included. Artificial or man-made structures such as rip-rap are also not included since the goal is to evaluate natural habitat.

Optimal – Over 70% of the stream reach has natural, stable habitat available for colonization by macroinvertebrates and/or fish. Four or more productive habitats are present. Deadfall, leaf litter, snags etc. are not new-fall but show evidence of decay. If less than four habitats are present drop to Suboptimal.

20 – Cobble riffle is the dominant habitat.

19 – Cobble run is the dominant habitat. Cobble riffles are present.

18 – Cobble run is the dominant habitat. Cobble riffles are not present.

17 – Productive habitat other than cobble riffle or run is dominant. Cobble riffles or runs are available.

16 – Productive habitat other than cobble riffle or run is dominant Cobble riffles and runs are absent.

Suboptimal – Natural, **s**table habitat covers 40 – 70% of stream reach. Three or more productive habitats present. If near 70% and more than three habitats are available go to optimal.

15 – Cobble riffle is the dominant habitat.

14 – Cobble run is the dominant habitat. Cobble riffles are present.

13 – Cobble run is the dominant habitat. Cobble riffles are not present.

12 - Habitat other than cobble riffle or run is dominant. Cobble riffles or runs are available.

11- Habitat other than cobble riffle or run is dominant Cobble riffles and runs are absent.

Marginal – Natural stable habitat covers 20 - 40% of stream reach **or** only 1 or 2 productive habitats are available in sufficient quantity to support a population. If coverage nears 40% and three or more productive habitats are present go to suboptimal.

10 - Cobble riffle is the dominant habitat.

9 - Cobble run is the dominant habitat. Cobble riffles are present.

8 - Cobble run is the dominant habitat. Cobble riffles are not present.

7 - Habitat other than cobble riffle or run is dominant. Cobble riffles or runs are available.

6 - Habitat other than cobble riffle or run is dominant Cobble riffles and runs are absent.

Poor – Less than 20% stable habitat regardless of number of habitats. Lack of habitat is obvious. Substrate unstable or lacking.

5 – At least two natural, stable, productive habitats are present in limited amount including either cobble riffles or runs.

4 – At least two natural, stable, productive habitats are present in limited amount. Cobble or riffle runs are absent.

3 – Cobble riffle or runs is the only habitat.

2 – Only one natural, stable, productive habitat is available. Cobble riffles or runs are absent.

1 – There are no natural, stable, productive habitats within the reach.

Comments: Use comment line to indicate what habitats are noticeably missing, or describe any additional factors which could affect interpretation of the score.

2. Embeddedness of riffles

Estimate the percent that rocks are covered or sunken into the silt, sand, or mud of the stream bottom. Ideally, observations should be done in cobble riffle areas. Ideally, riffles should have multiple layers of cobble loosely lying on each other providing niches for macroinvertebrates and fish between and under the rocks. Gravel riffles or cobble/gravel runs may be substituted if necessary. However, make sure riffles are not absent due to sedimentation (in which case the parameter should score 1).

In moderate to high gradient streams that naturally do not have cobble riffles (i.e. extremely high gradient boulder streams or some moderate gradient bedrock streams) the parameter would score lower due to lack of niche space even if embeddedness is not high.).
Two factors should be evaluated for this parameter.

To determine the condition category, estimate the amount to which the rock is surrounded by fine sediment. Fine sediments are silt, clay, sand, sludge etc. Discoloration on the bottom and sides of rocks is a good way to determine the percent of embeddedness. However, take care that additional cobble layers are not buried in sediment and are not visible.

To select the score within the category, examine the amount of niche space that is provided by layering of cobble (ideal). There should be lots of sediment free spaces between and under rocks for macroinvertebrates and small fish to live. If the stream type is not a cobble-riffle, other examples of riffle or run niches affected by embeddedness include the bottom area of round boulders where it curves into the substrate or the spaces between gravel in a bedrock fissure. In moderate gradient bedrock streams without gravel (for example those with bedrock shelves) examine loose rocks or slabs in areas of relatively fast flow. These are less productive and should be scored lower in the selected condition category.

Optimal: Gravel, cobble and boulders are 0 - 25% surrounded by fine sediment. If embeddedness is close to 25% use quality of niche space to differentiate between optimal and suboptimal condition categories. Optimal would be layered cobble. To determine the rank within this category, consider the available niche space.

20 – Niche spaces are free of sediment. Multiple layers of cobble provide niche space for colonization.

19 – Niche spaces are free of sediment but natural substrate does not provide multiple layers or is not cobble.

18 – Small amount of sediment (up to 10%) but niche spaces are not compromised. Multiple layers of cobble are available for colonization.

17 – Small amount of sediment (up to 10%) but niche spaces are not compromised. Natural substrate does not provide multiple layers.

16 – Sediment is more pronounced affecting up to 25% of niche space. Multiple layers of cobble are available for colonization.

Suboptimal: Gravel, cobble and boulders are 25% - 50% surrounded by fine sediment. If embeddedness is close to 25% use quality of niche space to differentiate between optimal and suboptimal condition categories. Optimal would be layered cobble. Likewise as number approaches 50% use quality of niche space to differentiate between suboptimal and marginal. Suboptimal would be layered cobble.

15 – Approximately 25% of niche space is affected. Substrate is not layered cobble.

14 – Approximately 30 - 35% of niche space affected. Substrate is layered cobble.

13 – Approximately 40 - 45% of niche space affected. Substrate is layered cobble.

12 – Approximately 30 - 45% of niche space affected. Substrate is not layered cobble

11 – Approximately 50% niche space is affected. Substrate is layered cobble.

Marginal: Gravel, cobble and boulders are 50% - 75% surrounded by fine sediment. As amount approaches 50%, use quality of niche space to differentiate between suboptimal and marginal condition categories. Suboptimal would be layered cobble.

10 – Approximately 50% of niche space affected. Substrate is not layered cobble.

9 – Approximately 55 - 65% of niche space affected. Substrate is layered cobble.

8 – Approximately 55 - 65% of niche space affected. Substrate is not layered cobble

7 – Approximately 70 - 75% of niche space affected. Substrate is layered cobble.

6 – Approximately 70 - 75% of niche space is affected. Substrate is not layered cobble.

Poor: Gravel, cobble and boulders are more than 75% surrounded by fine sediment.

5 – Approximately 80 - 85% of niche space affected. Substrate is layered cobble.

4 – Approximately 80 - 85% of niche space affected. Substrate is not layered cobble.

3 – Approximately 90 - 95% of niche space affected. Substrate is layered cobble

2 – Approximately 90 - 95% of niche space affected. Substrate is not layered cobble.

1 – Niche space is completely filled in by sediment.

Comments: Use comment line to describe type of sediment (sand, silt, clay, sludge etc.) and to describe any additional factors that would affect scoring.

3. Velocity/Depth Regime

Determine the patterns of velocity and depth. The four basic patterns are slow-deep, slow-shallow, fast-deep, and fast-shallow. The most productive streams will have all four patterns present. Differentiation between regimes will vary depending on stream size. Focus on habitat function. For example does the difference between fast-deep and fast-shallow in a small stream provide habitat for different taxa.

Condition Category is based on how many of the four regimes are present. Ranking is based on which ones are prevalent.

Optimal: All four velocity/depth regimes are present.

20 – All four velocity/depth regimes are equally available.

19 – Fast-shallow is the dominant regime.

18 – Slow-shallow is the dominant regime.

17 – Fast-deep is the dominant regime.

16 – Slow-deep is the dominant regime.

Suboptimal: Only 3 of the 4 velocity/depth regimes are present.

15 - Slow-deep is the only missing regime

14 - Fast-shallow is dominant

13 - Slow-shallow is dominant

12 - Fast-deep is dominant

11 – Slow deep is dominant.

Marginal: Only 2 of the 4 regimes are present. Both regimes are adequate to support aquatic population adapted to that habitat.

10 - Fast-Shallow and Slow-Shallow are present

9 - Fast-Shallow and Fast-Deep are present

8 - Fast-Shallow and Slow-Deep are present

7 - Slow-Shallow and Fast-Deep are present

6 - Fast-Deep and Slow-Deep are present.

Poor: One of the 4 regimes dominates the reach (if another is present it is too small or infrequent to sustain an aquatic population adapted to that habitat.

5 - Fast-Shallow is dominant a second regime may be present but is too infrequent to sustain a population.

4 - Slow-Shallow is dominant a second regime may be present but is too infrequent to sustain a population.

3 - Fast-Deep is dominant a second regime may be present but is too infrequent to sustain a population.

2 - Slow-Deep is dominant a second regime is present but is too infrequent to sustain a population.

1 - Slow-Deep is the only regime present.

Comments: Use the comment field to describe any additional factors that may affect scoring.

4. Sediment Deposition

This parameter is designed to measure the changes that have occurred to the stream bottom and flow patterns as a result of the deposition of small particles (gravel, sand, silt). It differs from embeddedness which is designed to measure loss of niche space.

Select condition category by estimating the percent of the stream bottom that is affected by sediment deposition. Areas of deposition occur in pools, bends, natural or man-made constrictions and other areas of slower flow. Deposition is also observable through the formation of islands, point bars (areas of increased deposition at the beginning of a meander that increase in size as the channel is diverted toward the outer bank) or shoals. Only areas of new, un-vegetated deposition on bars and islands should be considered when scoring.

Rank within each category is determined by the areas most affected by sediment deposition. Sediment in pools or slow areas will score higher than sediment on point bars and islands.

Optimal: Sediment deposition affects less than 5% of stream bottom in quiet areas. New deposition on islands and point bars is absent or minimal.

20 – No islands or point bars. No sediment in pools or slow areas.

19 – No new deposition on stable islands or point bars. No sediment in pools or slow areas.

18 - No new deposition on islands or point bars. Small amount of sediment in pools or slow areas.

17 – Slight amount of new deposition on islands or point bars. No sediment in pools or slow areas.

16 – Slight amount of new deposition on islands or point bars. Small amount of sediment in pools or slow areas. Almost 5% of bottom area affected.

Suboptimal: Sediment deposition affects 5 – 30% of stream bottom. Slight deposition in pool or slow areas. Some new deposition on islands and point bars.

15 – Sediment deposition affects 5 – 15% of the bottom substrate. Most of the deposition is in pools or bends with little new accumulation on islands or point bars.

14 – Sediment deposition affects 5 - 15% of the bottom substrate. Deposition occurs in both pool areas and as new accumulation on bars and islands.

13 – Sediment deposition affects 20 – 25% of the bottom substrate. Most of the deposition is in pools or bends with little new accumulation on islands or point bars.

12 – Sediment deposition affects 20 – 25% of the bottom substrate. Deposition occurs in both pool areas and as new accumulation on bars and islands.

11 – Sediment deposition affects 30% of the bottom substrate. The majority of deposition is in pools or bends with little new build-up of islands or point bars. Move to marginal if build-up of islands and point bars approaches 30%.

Marginal: Sediment deposition affects 30 – 50% of stream bottom. Sediment deposits at obstructions, constrictions and bends. Moderate deposition of pools.

10 – Sediment deposition affects 30% of stream bottom. Sediment deposits on bars and islands as well as pools and bends.

9 – Sediment deposition affects 35 – 45% of stream bottom. Most of deposition is in pools rather than build-up of bars and islands.

8 – Sediment deposition affects 35 – 45% of stream bottom. Moderate deposition of pools as well as new deposition on bars and islands.

7 – Sediment deposition affects almost half of the stream bottom. Most of deposition is in pools rather than new deposition on bars and islands.

6 – Sediment deposition affects almost half of the stream bottom. New sediment accumulation on bars and islands as well as in pools.

Poor: Heavy deposits of fine material. Increased bar development. More than 50% of the stream bottom changing frequently. Pools almost absent due to substantial sediment deposition.

5 – Approximately 50% of the bottom substrate is affected by sediment deposition.

4 – Approximately 60% of the bottom substrate is affected by sediment deposition.

3 - Approximately 70% of the bottom substrate is affected by sediment deposition.

2 – Approximately 80% of the bottom substrate is affected by sediment deposition.

1 – Sediment blankets stream bottom pools absent due to sediment deposition.

Comment: Use comment field if needed to describe other factors related to score.

5. Channel Flow Status

Condition category will be selected based on the amount of the streambed covered by water. Rank within the category will be determined by how much productive habitat is exposed. **If water has been backed up by obstructions (such as beaver dam, log jams, debris plugs) move assessment reach above or below the affected area**. If this is not possible, determine whether sampling is appropriate or should be postponed until conditions are more representative of actual stream conditions. Use comment field to explain if necessary. Assess flow status based on what is submerged during normal flow conditions, for example naturally exposed gravel beds do not indicate exposed habitat. Use comment field to note if flow is reduced due to natural low flow conditions, drought, irrigation, municipal water withdrawal, impoundment etc.

Optimal: Water reaches base of both lower banks and streambed is covered by water throughout the reach. Minimal amount of productive habitat is exposed. Riffle areas are fully submerged.

20 – Water is above the base of each bank. No productive habitats are exposed.

19 – Productive habitats including tree roots and riffles are submerged but some undercut areas may be above water. Riffle areas are fully submerged.

18 –Some tree roots are exposed but there is plenty of submerged root habitat available. Riffle areas are fully submerged. (If rooted bank habitat is naturally not present, score 16)

17 – If rooted bank habitat is present, some tree roots are exposed but there is plenty of submerged root habitat available Small areas of riffles may be minimally affected due to shallow water depth but riffle habitat is not compromised.

16 – Water reaches base of both banks and water still covers streambed. Root, riffle or other habitat is compromised due to water depth although is still available for colonization.

Suboptimal: Water covers more than 75% of the streambed but is less than 100% **or** 25% of productive habitat is exposed.

15 – One or more habitats may be absent due to water depth but riffle areas are not affected. (If productive riffle habitat is naturally not present score 11).

14 – Water depth in riffles is reduced but this has not affected size or frequency of riffles.

13 – Some riffle areas have become limited in size but none are totally exposed.

12 – A few smaller riffles have become exposed.

11 - Up to 25% of small riffles have become exposed or productive riffle habitat is not naturally available.

Marginal: Water covers 25% - 75% of the streambed, **and/or** stable habitat is mostly exposed.

10 – Waters covers 75% of channel. Most small riffles are exposed. (If productive riffle habitat is naturally not present score 11).

9 – Water covers 60 – 70% of streambed. All smaller riffles are exposed. Large riffles do not have significant exposed areas.

8 – Water covers about 50% of the streambed. Larger riffles are still present but are reduced in size.

7 – Water covers 30 – 40% of the streambed. Majority of riffle areas are exposed although small areas of largest riffles are still submerged.

6 – Water covers about 25% of streambed. Riffle areas are exposed although other rock habitat is available in run areas.

Poor: Very little water in channel and mostly present as standing pools. Little or no productive habitat due to lack of water.

5 – All riffles exposed. Runs extremely reduced. Very limited rock habitat available in running water.

4 – All riffles exposed. Runs reduced to trickles. No rock habitat available in running water.

3 – All riffles and runs exposed. Long stretches of pooled water provide some productive habitat. Stream may be flowing below surface between pools.

2 – Stream reduced to isolated pools with no productive habitat.

1 – Stream is dry

Comment: Use comment field to explain factors affecting the amount of water in the stream including natural (beaver activity, karst, drought etc.) and unnatural (dams, log jams at bridges, water withdrawal etc).

6. Channel Alteration

Determine how much, if at all, the stream reach has been altered by man-made activities (not beavers). Channel alteration is present when artificial embankments, riprap, and other forms of artificial bank stabilization or structures are present; when the stream is very straight for significant distances; when dams, culverts or bridges are present; when dredging or gravel/rock removal is evident, when snags/deadfall is removed, off-road vehicle activity has altered the bottom contours/compressed riffles and when other such artificial changes have occurred. Bridges, dams or other man-made structures upstream or downstream of the assessed reach should be considered if they affect flow patterns in the targeted reach.

Optimal: Channelization, gravel dredging, rock removal and off-road vehicle activity (past or present) absent or minimal. Stream has natural meander pattern. Shoring structures including riprap are absent. Artificial structures are not present in stream reach. Bridges, culverts, dams or other structures upstream or downstream are not affecting the stream reach.

20 – Stream has never been channelized and there are no artificial structures in stream reach or within impact area of reach. There is no evidence of past or present gravel dredging or rock removal. There is no evidence of off-road vehicle activity. Stream has normal meander pattern.

19 - Stream has never been channelized and there are no artificial structures in stream reach or within impact area of reach. Evidence of past rock removal is minimal. There is no evidence of gravel/sand dredging or 4-wheel activity. Stream flow pattern and habitat not affected.

18 – Stream has never been channelized and there are no artificial structures in stream reach or within impact area of reach. Evidence of past gravel/sand dredging is minimal. There is no evidence of 4-wheel activity. Stream flow pattern and habitat not affected.

17 – Past channel alteration in small area (less than 5% of reach). Stream flow pattern not affected. Modification is stable, well vegetated with natural vegetation, no erosion potential. There are no artificial structures in stream reach or within impact area. There is no evidence of 4-wheel activity.

16 – Evidence of past 4-wheel vehicle activity. Riffle and run areas intact, stream contours not affected. Artificial structures may be present outside of the reach but are not affecting the flow patterns, habitat or stream contours within reach.

Suboptimal: Evidence of channelization, dredging or 4-wheel activity up to 40%. May be longer reach if channelization is historic). Channel has stabilized and altered flow pattern does not affect colonization. Bridges, culverts, shoring or other artificial structures either within or outside of reach have not affected natural flow patterns.

15 – Historic channelization has stabilized (May also include pre-civil war rock walls.) Modification is stable, well vegetated with natural vegetation and no erosion potential.

14 – Bridge, culverts, shoring or artificial structures may be present but do not affect natural flow patterns in reach. (Includes structures upstream or downstream as well as within reach).

13 – Recent off-road vehicle activity in stream. Riffle or run areas slightly disturbed. Natural stabilization and re-colonization expected.

12 – Evidence of recent rock removal or gravel/sand dredging has had slight impact on reach. Natural stabilization and re-colonization is expected.

11- New channelization in up to 40% of stream reach. Modification is stable, well vegetated with natural vegetation, no erosion potential. (If not stable score 10).

Marginal: Channelization, dredging or 4-wheel activity 40 to 80% or less amount of channelization that has not stabilized. Bridges, culverts, shoring or other artificial structures either within or outside of reach may have slightly affected natural flow patterns.

10 – Less than 40% of reach altered but has not stabilized.

9 – 40 - 80% of reach has been channelized but is stable with natural vegetation.

8 – Bridge, culverts, shoring or artificial structures have slight affect on natural flow patterns in reach. (Includes structures upstream or downstream as well as within reach).

7 – Dredging, rock removal, 4-wheeling or other in-stream activity has impacted habitat in 40 - 80% of reach.

6 – 40 - 80% of reach has been altered and has not stabilized.

Poor: Over 80% of the stream reach channelized, dredged or affected by off-road vehicles, instream habitat greatly altered or removed entirely or artificial structures within reach or upstream/downstream of reach have greatly affected natural flow patterns.

5 – Over 80% of the stream reach has been channelized but is stable with natural vegetation.

4 – Over 80% of the stream reach is channelized and has been stabilized with artificial shoring.

3 – Over 80% of the stream reach is channelized and has not stabilized.

2 – Impoundment, bridge or other artificial structure has a high level of impact on normal stream flow and/or channel pattern. Include upstream or downstream structures that have seriously affected the sample reach.

1 – At least part of stream reach is in concrete or other artificial channel (including culverts).

Comment: Use comment field to indicate type of channel alteration (channelization, man-made dams, 4-wheel activity, construction vehicles). Also make note if beaver activity has altered stream (this is a natural condition so would score 20 if there are no artificial modifications but needs to be noted).

7. Frequency of Riffles, Bends or Other Re-Oxygenation Zones

Determine the pattern of stream morphology by estimating the sequencing of riffles. This is the only parameter where the hydrologic (not biological) definition of riffle will apply. Any swift moving re-oxygenation zones count, including bedrock riffles, large boulders, and bends. These areas provide diversity of habitat, control flow and provide refugia during storm events as well as re-oxygenate the water. To score this parameter, a longer segment may need to be incorporated into the evaluation if there are not at least 3 re-oxygenation areas within the sample reach. It may be necessary to pace off or measure distances. In larger streams where bends are the only re-oxygenation areas, maps may be used to determine frequency. Frequency will determine the condition category. Quality of habitat provided will determine the rank within the category.

Optimal: Occurrence of re-oxygenation zones relatively frequent. Distance between areas divided by average width of the stream <7:1.

20 – Re-oxygenation areas are high quality cobble riffles.

19 – Re-oxygenation areas are high quality gravel riffles.

18 – Re-oxygenation areas are not high quality cobble/gravel riffle but provide productive habitat (may include cobble runs or lower quality cobble riffles).

17 – Re-oxygenation areas are primarily bedrock, large boulder or other relatively unproductive habitat.

16 – Re-oxygenation areas are bends.

Suboptimal: Occurrence of re-oxygenation zones infrequent; distance between areas divided by average width of the stream is from 7 to 15.

15 – Re-oxygenation areas are high quality cobble riffles.

14 – Re-oxygenation areas are high quality gravel riffles.

13 – Re-oxygenation areas are not high quality cobble/gravel riffle but provide productive habitat (may include cobble runs or lower quality cobble riffles).

12 – Re-oxygenation areas are primarily bedrock, large boulder or other relatively unproductive habitat.

11 – Re-oxygenation areas are bends.

Marginal: Occasional re-oxygenation area. Distance between areas divided by average width of the stream is over 15 and up to 25.

10 – Re-oxygenation areas are high quality cobble riffles.

9 – Re-oxygenation areas are high quality gravel riffles.

8 – Re-oxygenation areas are not high quality cobble/gravel riffle but provide productive habitat (may include cobble runs or lower quality cobble riffles).

7 – Re-oxygenation areas are primarily bedrock, large boulder or other relatively unproductive habitat.

6 – Re-oxygenation areas are bends.

Poor: Generally all flat water or flat bedrock. Little opportunity for re-oxygenation. Distance between areas divided by average width of the stream is over 25.

5 – Re-oxygenation areas are high quality cobble riffles.

4 – Re-oxygenation areas are gravel riffles.

3 – Re-oxygenation areas are not high quality cobble/gravel riffle but provide productive habitat (may include cobble runs or lower quality cobble riffles).

2– Re-oxygenation areas are primarily bedrock, large boulder or other relatively unproductive habitat.

1 – Re-oxygenation areas are bends.

Comments: Use comment field to describe other factors affecting the score if needed such as atypical reoxygenation areas or poor quality riffles.

8. Bank Stability

Determine whether the stream banks are eroded or have the potential for erosion. Steep banks are more likely to collapse and suffer from erosion than are gently sloping banks, and are therefore considered less stable. Signs of erosion include crumbling, unvegetated banks, sloughing, exposed tree roots, and exposed soil. Each bank is evaluated separately on a scale of 0 to 10 and the cumulative score of both banks is used for this parameter. Left and right banks are determined by facing downstream.

Optimal: Banks stable, evidence of erosion or bank failure absent or minimal; little potential for future problems, < 5% of the bank affected.

10 – No signs of instability evident. Banks sloping. Little erosion potential.

9 – Some erosion evident or steep banks or some potential for erosion.

Suboptimal: Moderately stable, infrequent, small areas of erosion. 5 – 30% of bank in reach has areas of erosion.

8 – 5 - 15% of bank has areas of healed over erosion.

7 – 5 - 15% of bank has areas of erosion or other signs of instability. Some are not healed over.

6 – 20 - 30% of bank has areas of erosion or other signs of instability. If approaching 30%, score lower if banks are steep.

Marginal: Moderately unstable; 30 - 60% of bank in reach has areas of erosion or other signs of instability; high erosion potential during floods.

5 – 30 - 40% of bank has areas of erosion or other signs of instability. If approaching 40%, score lower if banks are steep.

4 – 40 - 50% of bank has areas of erosion or other signs of instability. If approaching 50%, score lower if banks are steep.

3 - 50 - 60% of bank has areas of erosion or other signs of instability. If approaching 60%, score lower if banks are steep or sloughing.

Poor: Unstable: many eroded areas; raw areas frequent along straight sections and bends; obvious bank sloughing; Over 60% of banks has areas of erosion or other signs of instability.

2 – 60 - 75% of bank has areas of erosion or other signs of instability.

1 – 80 - 90% of bank has areas of erosion or other signs of instability.

0 – There are no stable areas on bank.

Comment: Use comment field if needed to describe additional factors affecting scoring.

9. Bank Vegetative Protection

Determine the type and quality of vegetation on the stream bank. This is the area from the base of the bank to the top of the bank. The object is to determine the ability of the bank to resist erosion as well as the ability of the plants to uptake nutrients, control instream scouring, supply food to shredders and provide stream shading. Streams that have various classes of native vegetation providing full natural plant growth including groundcover, shrubs, understory trees and large trees will score highest.

In some regions, the introduction of exotics, such as kudzu, privet or honeysuckle, has virtually replaced all native vegetation. Although exotics may provide erosion control, they do not provide ideal food and habitat to stream organisms that have evolved to utilize native species. Banks that are dominated by non-native vegetation should score lower. A list of commonly encountered non-native species can be found in Appendix B. Species information, county distribution and pictures can be found at http://www.tneppc.org/Invasive_Exotic_Plant_List/The_List-by_habit.htm

Each bank is evaluated separately on a scale of 0 to 10 and the cumulative score of both banks is used for this parameter. Left and right banks are determined by facing downstream.

Condition category is determined by estimating the amount of bank covered by undisturbed native vegetation. Rank is determined by complexity of vegetation type.

Condition category is determined by estimating the amount of bank covered by undisturbed native vegetation. Rank is determined by complexity of vegetation type.

Optimal: More than 90% of the streambank surfaces and immediate riparian zone covered by undisturbed vegetation. All four classes (mature trees, understory trees, shrubs, groundcover) are represented. All plants allowed to grow naturally. All plants are native.

10 - No disruption. All classes of vegetation (mature trees, understory trees, shrubs, groundcover) are represented and allowed to grow naturally.

9 - Minimal disruption affecting less than 10% of stream bank. All classes of vegetation are represented and allowed to grow naturally.

Suboptimal: The majority (70 - 90%) of the bank is covered by undisturbed native vegetation. One class may not be not well represented. Disruption evident but not affecting full plant growth. Non-native vegetation may be present but rare (< 30%).

8 – Over 90% of bank area covered by native vegetation but one class not well represented.

7 – 70 - 90% of bank area covered by native vegetation. All classes of vegetation (mature trees, understory trees, shrubs, groundcover) are well represented and allowed to grow to full height.

6 – 70 - 90% of bank area covered by vegetation but one class not well represented. Other classes allowed to grow to full height. Non-natives may be present but do not affect natural vegetation growth

Marginal: 50 - 70% of the bank covered by undisturbed vegetation. Non-native vegetation may be common (30 - 50%). Two classes of vegetation may not be well represented.

5 – All classes of vegetation (mature trees, understory trees, shrubs, groundcover) are represented. If approaching 70% score suboptimal 7 if all vegetation allowed to grow to full height.

4 – One class of vegetation not well represented or not allowed to grow to full height. If approaching 70% score suboptimal 6 if all vegetation is native and allowed to grow to full height.

3 – Two classes of vegetation not well represented or not allowed to grow to full height or non-native vegetation is common.

Poor: Less than 50% of the bank is covered by undisturbed vegetation or more than two classes of vegetation are not well represented or most vegetation has been cropped. Non-native vegetation may be dominant (> 50%).

2 – Vegetation that is present is mostly native and allowed to grow to full height.

1 – Most vegetation is cropped and not allowed to grow to full height or non-native vegetation is dominant.

0 – Bank vegetation is absent or too sparse to provide bank protection or habitat.

Comment: Use the comment field to describe what class of plants are missing and/or describe non-native plants.

10. Riparian Vegetative Zone Width

Estimate the width of natural vegetation from the top of the stream bank out through the riparian zone (approximately 18 meters). Disturbance to the riparian zone occurs when there are roads, parking lots, fields, row crops, lawns, parks, picnic areas, bare soil, buildings, logging, campgrounds, golf courses, power-lines or other human activity.

Each bank is evaluated separately on a scale of 0 to 10 and the cumulative score of both banks is used for this parameter. Left and right banks are determined by facing downstream.

Condition category is determined by estimating the average width of the riparian zone from the top of the stream bank, outward. Generally the riparian ends at first indication of human disturbance with the exception of un-paved footpaths or trails in an otherwise undisturbed riparian.

Scoring within the category should be based on the level of impact the disturbance has. For example un-grazed fields would score higher than actively grazed fields. Lawns would score higher than paved areas.

Paths, and walkways in an otherwise undisturbed riparian zone may be judged to be minimal disturbance if they are unpaved, narrow and show no evidence of erosion. They should not affect condition category, but should lower score one point within category.

Optimal: Average width of riparian > 18 meters throughout reach.

10 – There is no human disturbance.

9 – Human disturbance minimal, for example, an un-paved footpath.

Sub-optimal: Average width of riparian 12 – 18 meters throughout reach.

8 – Human disturbance, after 12 meters of undisturbed riparian is minimal, for example an un-grazed hay field or areas of riparian that are less than 18 meters are small.

7 – Human disturbance, after 12 meters is vegetated but has frequent use or is close cropped. For example lawns, golf-courses, row crops, active pasture.

6 – Human disturbance, after 12 meters is not vegetated, for example paved or gravel lots, roads, bare dirt.

Marginal: Average width of riparian 6 – 11 meters throughout reach.

5 – Human disturbance, after 6 meters of undisturbed riparian is minimal, for example an un-grazed field, or areas that are less than 12 meters are small.

4 – Human disturbance, after 6 meters is vegetated but has frequent use or is close cropped. For example lawns, golf-courses, row crops, active pasture.

3– Human disturbance, after 6 meters is not vegetated, for example paved or gravel lots, roads, bare dirt.

Poor: Average width of riparian < 6 meters throughout reach.

2 – Human disturbance is minimal, for example an un-grazed field or areas that are less than 6 meters are small.

1 – Human disturbance is vegetated but has frequent use or is close cropped. For example lawns, golf-courses, row crops, active pasture.

0 – Human disturbance has removed all vegetation, for example paved or gravel lots, roads, bare dirt.

Comment field: Indicate type of disturbance and any additional factors affecting score.

Protocol D-2: Low Gradient Habitat Assessment Field Sheet.

The low gradient habitat field sheet (Appendix B) is used for low gradient streams. This will include streams in ecoregions 65abei, 73ab and 74b in west Tennessee as well as some streams in ecoregion 71i in middle Tennessee. This assessment may also be appropriate in lower reaches of larger streams in other ecoregions.

1. Epifaunal Substrate/Available Cover

When assessing this parameter, look at various types of natural structures available to macroinvertebrates and/or fish throughout the entire reach. Look for habitat that provides refugia, feeding, spawning or nursery functions. Do not count newly fallen trees, leaf litter that is not decaying or unstable habitats that will be washed out. Also do not include artificial habitat such as fish attractors, tires, appliances, rip-rap etc.

Habitats that are generally found in low gradient streams include:

Undercut banks
Submerged roots
Macrophyte beds
Submerged trees (not newfall)
Snags
Decaying leaf litter
Run rocks
Pool Rocks
Gravel riffles
Sediment
Bedrock fissures

To assign a condition category, first look at how much of the stream reach is covered by natural, stable, productive habitat. The numeric score (rank) within the condition category is assigned based on the variety and quality of the habitat. Variations in habitat that provide niches for different faunal types should be considered as different habitat types. For example, undercut banks with submerged tree roots should be considered separate from undercut banks with fine grassy roots.

Habitat that is not of sufficient quantity to provide faunal populations, does not show evidence of colonization (such as newly fallen leaves), is not productive (such as shifting sand) or is likely to wash out should not be included. Artificial structures such as rip-rap are also not included since the goal is to evaluate natural habitat.

Optimal – Over 50% of the stream reach has natural, stable habitat available for colonization by macroinvertebrates and/or fish. Three or more productive habitats are present. Deadfall, leaf litter, snags etc. are not new-fall but show evidence of decay. If less than three habitats are present drop to suboptimal.

20 – Deadfall and snags are the dominant habitat. At least two other habitats are available.

19 – Rooted banks are the dominant habitat. At least two other habitats are available.

18 – Macrophyte beds are the dominant habitat. At least two other habitats are available.

17 – Leaf litter is the dominant habitat. At least two other habitats are available.

16 – Another habitat is dominant. At least two other habitats are available.

Suboptimal – Natural stable habitat covers 30 - 50% of stream reach or less than three habitats are present. If nearing 30% and only one habitat is present, drop to marginal.

15 – Deadfall and snags are the dominant habitat

14 – Rooted banks are the dominant habitat

13 – Macrophytes beds are the dominant habitat.

12 – Leaf litter is the dominant habitat

11 – Another habitat is dominant.

Marginal – Natural stable habitat covers 10 – 30% of the stream reach. Availability less than desirable, substrate frequently disturbed or removed. Habitat diversity is reduced. If nearing 10% and only one habitat is available, drop to poor.

10 – Deadfall and snags are the dominant habitat.

9 – Rooted banks are the dominant habitat

8 – Macrophyte beds are the dominant habitat

7 – Leaf litter is the dominant habitat

6 – Another habitat is dominant.

Poor – Less than 10% stable habitat. Lack of habitat is obvious; substrate unstable or lacking.

5 – Rooted banks are the dominant habitat.

4 –.Deadfall and snags are the dominant habitat

3 – Macrophyte beds are the dominant habitat

2 – Leaf litter or another habitat is dominant.

1 – Habitat is lacking.

Comments: Use comment line to indicate what habitats are noticeably missing, or describe any additional factors which could affect interpretation of the score.

2. Channel Substrate Characterization (replaces Pool Substrate Characterization)

Evaluate the type and condition of the bottom substrate in the channel. Firmer sediment such as gravel, firm sand, and rooted aquatic plants support a wider variety of organisms and should be scored higher than a substrate dominated by soft sand, mud or bedrock with no plants. In addition, a stream that has a uniform substrate will support fewer types of organisms and should score lower than a stream that has a variety of substrate type. Root mats for this parameter are those within the bottom substrate of the channel and should not be confused with rooted undercut banks. Firm sand is desirable while soft sand will score lower. Fissured bedrock with crevices and rock shelves will score higher than smooth bedrock.

The type of substrate will determine the condition category. Rank within the category will be based on the ratio of substrate type.

Optimal – Good mixture of substrate materials with gravel and firm sand prevalent. Root mats and submerged vegetation are common.

20 – Even mix of gravel and firm sand. Both root mats and submerged vegetation are common.

19 – Mixture of substrate including firm sand. Gravel is dominant. Both root mats and submerged vegetation are common.

18 – Mixture of substrate including firm sand. Gravel is dominant. Either root mats or submerged vegetation is missing.

17 – Mixture of substrate including gravel. Firm sand is dominant. Both root mats and submerged vegetation are common.

16 – Mixture of substrate including gravel. Firm sand is dominant. Either root mats or submerged vegetation is missing.

Suboptimal - Mixture of soft sand, mud or clay. Substrate may also be fissured bedrock. Some root mats and submerged vegetation present.

15 – Mixture of soft sand, mud and clay. No substrate dominant. Both root mats and submerged vegetation present.

14 – Mixture of soft sand, mud and clay, mud dominant. Both root mats and submerged vegetation present.

13 – Mixture of soft sand and mud, mud dominant. Either root mats or submerged vegetation is missing.

12 – Mixture of soft sand and clay or substrate is fissured bedrock with frequent fissures and shelves. Some root mats and submerged vegetation present.

11 – Mixture of soft sand and clay or substrate is fissured bedrock with frequent fissures and shelves. Either root mats or submerged vegetation is missing.

Marginal – All mud, clay or soft sand bottom; substrate may also be fissured bedrock; little or no root mat; no submerged vegetation present.

10 – Mud bottom, some root mat present.

9 – Soft Sand bottom, some root mat present.

8 – Clay bottom, some root mat present.

7 – Mud or fissured bedrock bottom, no root mat present.

6 – Soft sand or clay bottom, no root mat present.

Poor – Hard-pan clay, conglomerate or flat bedrock; no root mat or vegetation.

5 – Predominantly flat bedrock, other non-bedrock substrate available.

4 – Predominantly flat bedrock, infrequent crevices and/or shelves provide some habitat.

3 – Predominantly conglomerate substrate.

2 – Predominantly flat bedrock substrate.

1 – Predominantly hard-pan clay substrate.

Comments: Use comment field if needed to clarify scoring or describe substrate.

3. Pool Variability

Rate the overall mixture of pool types found in the stream, according to size and depth (this will vary depending on the size of the stream). The four basic types of pools are large-shallow, large-deep, small-shallow, and small-deep. A stream having many different pool types will support a wider variety of aquatic species and should score higher. The variety of pool types will determine condition category. The quality of these pools will determine rank within the category.

Optimal: Even mix of large-shallow, large–deep, small-shallow, small-deep pools present.

20 – Some pools are at least 1 meter deep and are of sufficient length to support fish populations.

19 – Large-deep pools are less than 1 meter but are of sufficient size and depth to support fish populations.

18 – Large-deep pools are at least 1 meter providing distinct habitat but are of insufficient length to support fish populations.

17 – Smaller stream, deep pools provide distinct benthic habitat from shallow pools but are not of sufficient depth to support fish populations.

16 – Although all 4 pool types are available, some may not provide distinct faunal habitat due to small stream size.

Sub-optimal: Majority of pools large-deep; very few shallow.

15 – Some pools are at least 1 meter deep and are of sufficient length to support fish populations.

14 – Large-deep pools are less than 1 meter but are of sufficient size and depth to support fish populations.

13 – Large-deep pools are at least 1 meter providing distinct benthic habitat but are of insufficient length to support fish populations.

12 – Smaller stream, deep pools provide distinct benthic habitat but are not of sufficient depth to support fish populations.

11 – Smaller stream, deep pools though present may not provide distinct habitat.

Marginal: Shallow pools much more prevalent than deep pools.

10 – Some pools are at least 1 meter deep and are of sufficient length to support fish populations.

9 – Large-deep pools are less than 1 meter but are of sufficient size and depth to support fish populations.

8 – Large-deep pools are at least 1 meter providing distinct habitat but are of insufficient length to support fish populations.

7 – Smaller stream, deep pools are less than 1 meter and are of insufficient size to support fish populations but do provide distinct benthic habitat from shallow pools.

6 – Smaller stream, deep pools though present may not be frequent enough or of sufficient size to provide distinct benthic habitat from shallow pools.

Poor: Majority of pools small-shallow or pools absent

5 – Both large-shallow and small-shallow pools present.

4 – Only small-shallow pools present.

3 – Pools absent, although slow current areas are present.

2 – Pools absent, although there are depth changes within channel.

1 – Channel is a continuous run with little or no changes in velocity or depth.

Comments: Use the comment field if needed for clarification or to describe atypical characteristics affecting scoring.

4. Sediment deposition

This parameter is designed to measure the changes that have occurred to the stream bottom and flow patterns as a result of the deposition of small particles (gravel, sand, silt).

Select condition category by estimating the percent of the stream bottom that is affected by sediment deposition. Areas of deposition occur in pools, bends, natural or man-made constrictions and other areas of slower flow. Deposition is also observable through the formation of islands, point bars (areas of increased deposition at the beginning of a meander that increase in size as the channel is diverted toward the outer bank) or shoals. Only areas of new, un-vegetated deposition on bars and islands should be considered when scoring.

Rank within each category is determined by the areas most affected by sediment deposition. Sediment in pools or slow areas will score higher than sediment on point bars and islands.

Optimal: Sediment deposition affects less than 20% of stream bottom in quiet areas. New deposition on islands and point bars is absent or minimal.

20 – No islands or point bars. No sediment in pools or slow areas.

19 – No new deposition on stable islands or point bars. No sediment in pools or slow areas.

18 – No new deposition on islands or point bars. Small amount of sediment in pools or slow areas.

17 – Small amount of new deposition on islands or point bars. No sediment in pools or slow areas.

16 – Small amount of new deposition on islands or point bars. Small amount of sediment in pools or slow areas. Up to 20% of bottom area affected. (As deposition approaches 20% if most of deposition is an increase in island or bars drop to suboptimal.)

Suboptimal: Some new increase in bar formation, mostly from gravel, sand or fine sediment (20 - 50%) of the bottom affected. Slight deposition in pools.

15 – Sediment deposition affects 20 - 30% of the bottom substrate. Most of the deposition is in pools or bends with little new accumulation on islands or point bars.

14 – Sediment deposition affects 20 - 30% of the bottom substrate. Deposition occurs in both pool areas and as new accumulation on bars and islands.

13 – Sediment deposition affects 35 - 45% of the bottom substrate. Most of the deposition is in pools or bends with little new accumulation on islands or point bars.

12 – Sediment deposition affects 35 - 45% of the bottom substrate. Deposition occurs in both pool areas and as new accumulation on bars and islands.

11 – Sediment deposition affects 50% of the bottom substrate. Deposition occurs primarily on pool areas. As new accumulation on bars and islands approaches 50% drop to marginal.

Marginal: Moderate deposition of new gravel, sand or fine sediment on old and new bars 50-80% of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent.

10 – Sediment deposition affects 50% of stream bottom. Sediment deposits on bars and islands as well as pools and bends.

9 – Sediment deposition affects 55 – 65% of stream bottom. Most of deposition is in pools rather than build-up of bars and islands.

8 – Sediment deposition affects 55 – 65% of stream bottom. Moderate deposition of pools as well as new deposition on bars and islands.

7 – Sediment deposition affects 70 - 80% of the stream bottom. Most of deposition is in pools rather than new deposition on bars and islands.

6 – Sediment deposition affects 70 - 80% of the stream bottom. New sediment accumulation on bars and islands as well as in pools.

Poor: Heavy deposits of fine material, increased bar development; more than 80% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.

5 – Approximately 85 - 90% of the bottom substrate is affected by sediment deposition. Pools heavily affected but still present or pools naturally absent.

4 – Approximately 85- 90% of the bottom substrate is affected by sediment deposition. Pools absent due to sediment deposition.

3 - Approximately 95% of the bottom substrate is affected by sediment deposition. Pools heavily affected but still present or pools naturally absent.

2 – Approximately 95% of the bottom substrate is affected by sediment deposition. Pools absent due to sediment deposition.

1 – Sediment blankets 100% of stream bottom.

Comment: Use comment field if needed to describe other factors related to score.

5. Channel Flow Status

Estimate the degree to which the channel is filled with water. Condition category will be selected based on amount of streambed covered. Rank within the category will be determined by how much productive habitat is exposed. If the stream has no habitat, score lowest rank within condition category and explain in comments. If water has been backed up by obstructions (such as beaver dam, log jams, bedrock during low flow) move assessment reach above or below the affected area or consider postponing sampling until an accurate assessment of stream conditions can be achieved. Assess flow status based on what is submerged during normal flow conditions, for example naturally exposed gravel beds do not indicate exposed habitat. Use comment field to note if flow is reduced due to natural low flow conditions, drought, irrigation, municipal water withdrawal, impoundment etc.

Optimal: Water reaches base of both lower banks throughout reach and covers stream bed. Minimal productive habitat is exposed.

20 – Water is above the base of each bank and no productive habitats are exposed.

19 – Roots are submerged but some undercut areas may be above water. Other productive habitats are not affected.

18 – Some shallow roots are exposed but there is plenty of submerged root habitat available. Other habitats are not affected.

17 – Most shallow roots are exposed, but there is plenty of submerged root habitat. Other habitats are not affected.

16 – Some larger rooted areas are partially exposed but there is plenty of submerged root habitat. Other productive habitats are not affected.

Suboptimal: Water covers more than 75% of the streambed and less than 25% of productive habitat is exposed.

15 – Some submerged rooted areas are totally exposed although the habitat is still plentiful. Other productive habitats are not affected.

14 – Most root habitat is exposed. Other productive habitats are available.

13 – All root habitat is exposed. Other productive habitats are available.

12 – A second near-shore habitat such as macrophyte beds is partially exposed. Other, mid channel habitats such as fallen trees are available for full colonization.

11 – All near shore habitat is compromised although some is available. Other, mid-channel habitats such as fallen trees are available for full colonization.

Marginal: Water covers 25% - 75% of the streambed, and/or productive habitat is mostly exposed.

10 – Waters covers about 75% of streambed. All near shore habitat is exposed. Mid channel habitats are available and not affected.

9 – Water covers 60 – 70% of streambed. All near shore habitat is exposed. Some mid channel habitat such as fallen trees and snags are compromised but still available for full colonization.

8 – Water covers about 50% of streambed. Mid channel habitat is compromised but at least one productive habitat is still common.

7 – Water covers 30 – 40% of streambed. Most habitat is exposed, at least one productive habitat is still common.

6 – Water covers about 25% of streambed. Isolated areas of productive habitat.

Poor: Very little water in channel and mostly present as standing pools. Little or no productive habitat due to lack of water.

5 – Very little flow evident. Isolated patches of productive habitat.

4 – Very little flow evident. Remaining habitat is un-productive

3 – Water reduced to standing pools. Isolated patches of productive habitat.

2 – Water reduced to standing pools. Remaining habitat is un-productive.

1 – Stream is dry.

Comment: Use comment field to explain factors affecting the amount of water in the stream including natural (beaver activity, karst, drought etc.) and unnatural (man-made dams, log jams at bridges, water withdrawal etc). Also use comment field to indicate if there is naturally no productive habitat which would affect ranking within category.

6. Channel Alteration

Determine how much, if at all, the stream reach has been altered by man-made activities (not beavers). Channel alteration is present when artificial embankments, riprap, and other forms of artificial bank stabilization or structures are present; when the stream is very straight for significant distances; when dams, culverts or bridges are present; when dredging or gravel/rock removal is evident, when snags/deadfall is removed, 4-wheel activity has altered the bottom contours/compressed riffles and when other such artificial changes have occurred. Bridges, dams or other man-made structures upstream or downstream of the assessed reach should be considered if they affect flow patterns in the targeted reach.

Optimal: Channelization, dredging, or 4-wheel activity (past or present) absent or minimal. Stream has normal meander pattern. Shoring structures including riprap are absent. Artificial structures are not present in stream reach. Bridges, culverts, dams or other structures upstream or downstream are not affecting the stream reach.

20 – Stream has never been channelized and there are no artificial structures in stream reach or within impact area of reach. There is no evidence of past or present dredging or rock removal. There is no evidence of 4-wheel activity. Stream has normal meander pattern.

19 – Stream has never been channelized and there are no artificial structures in stream reach or within impact area of reach. Evidence of past rock removal. There is no evidence of gravel/sand dredging or 4-wheel activity. Stream flow pattern and habitat not affected.

18 – Stream has never been channelized and there are no artificial structures in stream reach or within impact area of reach. Evidence of past gravel or sand dredging. There is no evidence of 4-wheel activity. Stream flow pattern and habitat not affected.

17 – Past channel alteration in small area (less than 5% of reach). Stream flow pattern not affected. Modification is stable, well vegetated with natural vegetation, no erosion potential. There are no artificial structures in stream reach or within impact area.

16 – Evidence of recent 4-wheel activity. In-stream habitat, stream contours and banks not affected. Artificial structures may be present outside of the reach but are not affecting the flow patterns, habitat or stream contours within reach.

Suboptimal: Channelization, dredging or 4-wheel activity up to 40%. May be longer reach if channelization is historic. Channelization has stabilized and altered flow pattern does not affect colonization. Bridges, culverts, shoring or other artificial structures either within or outside of reach have not affected natural flow patterns.

15 – Historic channelization has stabilized. Modification is stable, well vegetated with natural vegetation and no erosion potential.

14 –- Bridge, culverts, shoring or artificial structures may be present but do not affect natural flow patterns in reach. (Includes structures upstream or downstream as well as within reach.)

13 – Recent off-road vehicle activity in stream. Channel substrate slightly disturbed. Natural stabilization and re-colonization expected.

12 – Evidence of recent rock removal or gravel/sand dredging has had slight impact on reach. Natural stabilization and re-colonization is expected.

11 – New channelization in up to 40% of stream reach. Modification is stable, well vegetated with natural vegetation, no erosion potential. (If not stable score 10.)

Marginal: Channelization, dredging or 4-wheel activity 40 - 80% or less amount of channelization that has not stabilized. Bridges, culverts, shoring or other artificial structures either within or outside of reach may have slightly affected natural flow patterns.

10 – Less than 40% of reach altered but has not stabilized.

9 – 40 - 80% of reach has been recently been channelized but is stable with natural vegetation.

8 – Bridge, culverts, shoring or artificial structures have slight affect on natural flow patterns in reach. (Includes structures upstream or downstream as well as within reach.)

7 – Dredging, rock removal, 4-wheeling or other in-stream activity has impacted habitat in 40 - 80% of reach.

6 – 40 - 80% of reach has been altered and has not stabilized.

Poor: Over 80% of the stream reach channelized, dredged or affected by 4-wheel activity, instream habitat greatly altered or removed entirely or artificial structures within reach or upstream/downstream of reach have greatly affected natural flow patterns.

5 – Over 80% of the stream reach has recently been channelized but is stable with natural vegetation.

4 – Over 80% of the stream reach is channelized and has been stabilized with artificial shoring.

3 – Over 80% of the stream reach is channelized and has not stabilized.

2 – Impoundment, bridge or other artificial structure has a high level of impact on normal stream flow and/or channel pattern. Include upstream or downstream structures that have substantially affected the sample reach.

1 – At least part of stream reach is in concrete or other artificial channel (including culverts).

Comment: Use comment field to indicate type of channel alteration (channelization, man-made dams, 4-wheel activity) and to explain any score adjustments. Also make note if beaver activity has altered stream (this is a natural condition so would score 20 if there are no artificial modifications but needs to be noted).

7. Channel Sinuosity

Evaluate the meandering or sinuosity of the stream. A high degree of sinuosity provides diverse habitat for macroinvertebrates and the stream is better able to handle surges when the flow fluctuates due to rain events. **To estimate this parameter, a longer segment or reach than that designated for the sampling should be incorporated into the evaluation. This will vary by site, but should include at least 2 bends. Maps may be used to estimate the sinuosity of larger streams where field evaluations are not practical.**

The amount the meanders increase stream length determines the condition category. The quality of the meander (whether additional macroinvertebrate or habitat is provided) determines the rank.

Optimal – The bends in the stream increase the stream length 3-4 times longer than if it was in a straight line.

20 – Stream meander increases stream length more than 4 times longer than a straight line.

19 – Stream meander increases stream length 4 times longer than a straight line.

18 – Stream meander increases stream length 3.5 times longer than a straight line. Bends provide productive macroinvertebrate habitat.

17 – Stream meander increases stream length 3.5 times longer than a straight line. Bends do not do not provide additional macroinvertebrate habitat.

16 – Stream meander increases stream length 3 times longer than a straight line. Bends provide productive macroinvertebrate habitat.

Suboptimal - The bends in the stream increase the stream length 2-3 times longer than if it was in a straight line.

15 – Stream meander increases stream length 3 times longer than a straight line. Bends do not provide additional macroinvertebrate habitat.

14 – Stream meander increases stream length 2.5 times longer than a straight line. Bends provide productive macroinvertebrate habitat.

13 – Stream meanders increase stream length 2.5 times longer than a straight line. Bends do not provide additional macroinvertebrate habitat.

12 – Stream meander increases stream length 2 times longer than a straight line. Bends provide productive macroinvertebrate habitat.

11 – Stream meander increases stream length 2 times longer than a straight line. Bends do not provide additional macroinvertebrate habitat.

Marginal – The bends in the stream increase the stream length 1-2 times longer than if it was in a straight line.

10 – Stream meander increases stream length 2 times longer than a straight line. Bends provide additional macroinvertebrate habitat.

9 – Stream meander increases stream length 1.5 times longer than a straight line. Bends provide productive macroinvertebrate habitat.

8 – Stream meanders increase stream length 1.5 times longer than a straight line. Bends do not provide additional macroinvertebrate habitat.

7 – Stream meander increases stream length 1 times longer than a straight line. Bends provide productive macroinvertebrate habitat.

6 – Stream meander increases stream length 1 times longer than a straight line. Bends do not provide additional macroinvertebrate habitat.

Poor – Channel straight; waterway has been channelized for a long distance.

5 – Straight channel offset by some slight curves which, not meanders, do serve to provide some habitat and some energy dissipation during surges.

4 – Straight channel with more than one slight curve.

3 – Straight channel with a single slight curve.

2 – Straight channel with no curves but some bank indentations providing habitat. (Stable indentations not subject to erosion).

1 – Channel completely straight with no curves or stable indentations.

Comments: Use comment field if necessary to describe any other factors that influenced scoring.

8. Bank Stability

Determine whether the stream banks are eroded or have the potential for erosion. Steep banks are more likely to collapse and suffer from erosion than are gently sloping banks, and are therefore considered less stable. Signs of instability include crumbling, unvegetated banks, exposed tree roots, slumping and/or exposed soil. Each bank is evaluated separately on a scale of 0 to 10 and the cumulative score of both banks is used for this parameter. Left and right banks are determined by facing downstream.

Optimal: Banks stable, evidence of erosion or bank failure absent or minimal; little potential for future problems, < 5% of the bank affected.

10 – No signs of instability evident. Banks sloping. Little erosion potential.

9 – Some erosion evident or steep banks or some potential for erosion.

Suboptimal: Moderately stable, infrequent, small areas of erosion. 5 – 30% of bank in reach has areas of erosion or other signs of instability.

8 – 5 - 15% of bank has areas of healed over erosion.

7 – 5 - 15% of bank has areas of erosion or other signs of instability. Some are not healed over.

6 – 20 - 30% of bank has areas of erosion or other signs of instability. If approaching 30%, score lower if banks are steep.

Marginal: Moderately unstable; 30-60% of bank in reach has areas of erosion or other signs of instability; high erosion potential during floods.

5 – 30 - 40% of bank has areas of erosion or other signs of instability. If approaching 40%, score lower if banks are steep.

4 – 40 - 50% of bank has areas of erosion or other signs of instability. If approaching 50%, score lower if banks are steep.

3 – 50 - 60% of bank has areas of erosion or other signs of instability If approaching 60%, score lower if banks are steep or sloughing.

Poor: Unstable: many eroded areas; raw areas frequent along straight sections and bends; obvious bank sloughing; Over 60% of banks has areas of erosion or other signs of instability.

2 – 60 - 75% of bank has areas of erosion or other signs of instability.

1 – 80 - 90% of bank has areas of erosion or other signs of instability.

0 – There are no stable areas on bank.

Comment: Use comment field if needed to describe additional factors affecting scoring.

9. Bank Vegetative Protection

Determine the type and quality of vegetation on the stream bank. This is the area from the base of the bank to the top of the bank. The object is to determine the ability of the bank to resist erosion as well as the ability of the plants to uptake nutrients, control instream scouring, supply food to shredders and provide stream shading. Streams that have various classes of native vegetation providing full natural plant growth including groundcover, shrubs, understory trees and mature trees will score highest.

In some regions, the introduction of exotics, such as kudzu or privet, has virtually replaced all native vegetation. Although exotics may provide erosion control, they do not provide ideal food and habitat to stream organisms that have evolved to utilize native species. Banks that are dominated by non-native vegetation should score lower. A list of commonly encountered non-native species can be found in Appendix B. Species information and links to county distribution and field guides can be found at http://www.tneppc.org/Invasive_Exotic_Plant_List/The_List-by_habit.htm

Each bank is evaluated separately on a scale of 0 to 10 and the cumulative score of both banks is used for this parameter. Left and right banks are determined by facing downstream.

Condition category is determined by estimating the amount of bank covered by **undisturbed native vegetation**. Rank is determined by complexity of vegetation type.

Optimal: More than 90% of the streambank surfaces and immediate riparian zone covered by undisturbed vegetation. All four classes (mature trees, understory trees, shrubs, groundcover) are represented. All plants allowed to grow naturally. All plants are native.

10 - No disruption. All classes of vegetation (mature trees, understory trees, shrubs, groundcover) are represented and allowed to grow naturally.

9 - Minimal disruption affecting less than 10% of stream bank. All classes of vegetation are represented and allowed to grow naturally.

Suboptimal: The majority (70 - 90%) of the bank is covered by undisturbed native vegetation. One class may not be not well represented. Disruption evident but not affecting full plant growth. Non-native vegetation may be present but rare (< 30%).

8 – Over 90% of bank area covered by native vegetation but one class not well represented.

7 – 70-90% of bank area covered by native vegetation. All classes of vegetation (mature trees, understory trees, shrubs, groundcover) are well represented and allowed to grow to full height.

6 – 70 - 90% of bank area covered by native vegetation but one class not well represented. Other classes allowed to grow to full height.

Marginal: 50 - 70% of the bank covered by undisturbed vegetation. Non-native vegetation may be common (30 - 50%). Two classes of vegetation may not be well represented.

5 – All classes of vegetation (mature trees, understory trees, shrubs, groundcover) are represented. If approaching 70% score suboptimal 7 if all vegetation allowed to grow to full height.

4 – One class of vegetation not well represented or not allowed to grow to full height. If approaching 70% score suboptimal 6 if all vegetation is native and allowed to grow to full height.

3 – Two classes of vegetation not well represented or not allowed to grow to full height or non-native vegetation is common.

Poor: Less than 50% of the bank is covered by undisturbed vegetation or more than two classes of vegetation are not well represented or most vegetation has been cropped. Non-native vegetation may be dominant (> 50%).

2 – Vegetation that is present is mostly native and allowed to grow to full height.

1 – Most vegetation is not allowed to grow to full height or is non-native.

0 – Bank vegetation is absent or too sparse to provide bank protection or habitat.

Comment: Use the comment field to describe what class of plants are missing and/or describe exotic plants.

10. Riparian Vegetative Zone Width

Estimate the width of natural vegetation from the top of the stream bank out through the riparian zone (approximately 18 meters). Disturbance to the riparian zone occurs when there are roads, parking lots, fields, row crops, lawns, parks, bare soil, buildings, logging, campgrounds, golf courses or other human activity.

Each bank is evaluated separately on a scale of 0 to 10 and the cumulative score of both banks is used for this parameter. Left and right banks are determined by facing downstream.

Condition category is determined by estimating the width of the riparian zone from the top of the stream bank, outward. Generally the riparian ends at first indication of human disturbance with the exception of un-paved footpaths or trails in an otherwise undisturbed riparian.

Scoring within the category should be based on the level of impact the disturbance has. For example un-grazed fields would score higher than active fields. Lawns would score higher than paved areas.

Paths, and walkways in an otherwise undisturbed riparian zone may be judged to be minimal disturbance if they are narrow, unpaved and show no evidence of erosion. They should not affect condition category, but should lower score one point within category.

Optimal: Average width of riparian > 18 meters throughout reach.

10 – There is no human disturbance.

9 – Human disturbance minimal, for example, an un-paved footpath.

Sub-optimal: Average width of riparian 12 – 18 meters throughout reach.

8 – Human disturbance, after 12 meters of undisturbed riparian is minimal, for example an un-grazed hay field or areas of riparian that are less than 18 meters are small.

7 – Human disturbance, after 12 meters is vegetated but has frequent use or is close cropped. For example lawns, golf-courses, row crops, active pasture.

6 – Human disturbance, after 12 meters is not vegetated, for example paved or gravel lots, roads, bare dirt.

Marginal: Average width of riparian 6 – 11 meters throughout reach.

5 – Human disturbance, after 6 meters of undisturbed riparian is minimal, for example an un-grazed field, or areas that are less than 12 meters are small.

4 – Human disturbance, after 6 meters is vegetated but has frequent use or is close cropped. For example lawns, golf-courses, row crops, active pasture.

3 – Human disturbance, after 6 meters is not vegetated, for example paved or gravel lots, roads, bare dirt.

Poor: Average width of riparian < 6 meters throughout reach.

2 – Human disturbance is minimal, for example an un-grazed field or areas that are less than 6 meters are small.

1 – Human disturbance is vegetated but has frequent use or is close cropped. For example lawns, golf-courses, row crops, active pasture.

0 – Human disturbance has removed all vegetation, for example paved or gravel lots, roads, bare dirt.

Comment field: Indicate type of disturbance and any additional factors affecting score.

Scoring

Total the 10 habitat parameters and compare the score to the Habitat Assessment Guidelines (Table 2) to determine whether the habitat is capable of supporting a healthy benthic community (not impaired). Note that habitat scores in four ecoregions (68a, 68c, 69d and 69e) vary by season. Indicate at bottom of field sheet if total habitat score is above (not impaired) or below (impaired) ecoregional guidelines in blank provided. If score is low indicate whether this is a result of natural conditions (such as drought or beaver activity) or is the result of human disturbance. Write a brief description in space provided. (If more room is needed attach another sheet).

Sometimes it may be useful to evaluate individual parameters in addition to the total habitat score. For example even if the total habitat score meets regional guidelines, the individual parameters of embeddedness and sediment deposition may be low indicating a problem with sedimentation. Likewise, there may be a problem with riparian removal even though habitat scores meet regional guidelines. On the other hand, a low total score may not indicate a habitat problem if the channel flow status and velocity depth regime score low in a region where reference streams have extremely reduced flow during the summer and fall. Appendix A provides ecoregion specific expectations for each parameter on the Habitat guidelines field sheet.

Table 2: Habitat Assessment Guidelines

Ecoregion	Habitat Field sheet	Season	Streams > 2 sq mile drainage Not Impaired	Headwater streams ≤ 2 square mile drainage Not impaired
65a	Low Grad.	Jan. – Dec.	≥ 118	≥ 115
65b	Low Grad.	Jan. – Dec.	≥ 118	≥ 115
65e	Low Grad.	Jan. – Dec.	≥ 129	≥ 115
65i	Low Grad.	Jan. – Dec.	≥ 118	≥ 115
65j	High Grad.	Jan. – Dec.	≥ 140	≥ 140
66d	High Grad.	Jan. – Dec.	≥ 157	≥ 157
66e	High Grad.	Jan. – Dec.	≥ 152	≥ 151
66f	High Grad.	Jan. – Dec.	≥ 144	≥ 144
66g	High Grad.	Jan. – Dec.	≥ 147	≥ 137
67f	High Grad.	Jan. – Dec.	≥ 140	≥ 135
67g	High Grad.	Jan. – Dec.	≥ 123	≥ 138
67h	High Grad.	Jan. – Dec.	≥ 128	≥ 133
67i	High Grad.	Jan. – Dec.	≥ 119	≥ 119
68a	High Grad.	Jan. – June	≥ 142	≥ 132
68a	High Grad.	July – Dec.	≥ 144	≥ 143
68b	High Grad.	Jan. – Dec	≥ 123	≥ 122
68c	High Grad.	Jan. – June	≥ 158	≥ 154
68c	High Grad.	July – Dec.	≥ 139	≥ 133
69d	High Grad.	Jan. – June	≥ 139	≥ 137
69d	High Grad.	July – Dec.	≥ 133	≥ 127
69e	High Grad.	Jan. - June	≥ 139	≥ 137
69e	High Grad.	July – Dec.	≥ 135	≥ 127
71e	High Grad.	Jan. – Dec.	≥ 121	≥ 120
71f	High Grad.	Jan. – Dec.	≥ 128	≥ 133
71g	High Grad.	Jan. – Dec.	≥ 134	≥ 140
71h	High Grad.	Jan. – Dec.	≥ 127	≥ 132
71i	High Grad.	Jan. – Dec	≥ 121	≥ 119
71i	Low Grad.	Jan – Dec	≥ 111	≥ 111
73a	Low Grad.	Jan. – Dec.	≥ 114	≥ 114
74a	High Grad.	Jan. – Dec.	≥ 117	≥ 111
74b	Low Grad.	Jan. – Dec.	≥ 111	≥ 113

Protocol E: Stream Survey Field sheet

Biologist/Environmental Specialist

The stream survey field sheet must be completed when macroinvertebrate surveys are done (Appendix B). Information on the field sheet is designed to help make assessment decisions and provide supplemental information for interpreting biological sample results. Add additional information, not included on the field sheet, as needed. Only use the version provided in Appendix B. Earlier versions should no longer be used. Consult all personnel present during sampling for additional observations that may have been overlooked before leaving the site. It is recommended that the field sheet be copied on waterproof paper.

1. Stream Survey Information

   Complete all information in this section. Lat/long does not need to be completed on existing sites provided the rest of the information is in the water quality database.

   a. **Station ID** as already assigned in Water Quality Database or following protocol B for new stations. (Do not rely on memory or assume no-one else has ever collected any type of sample at this location. TVA or a permit holder may already have a station at this location and a TDEC station ID has been established. Often, a site was collected by another team from the same field office or several years ago. It is important that consistent IDs be used for all data associated with a site no matter who collected it.)
   b. **Stream Name** based on 24K scale topographic map or GIS layer.
   c. **Station Location**. If it is an existing station, use location in current stations table of the water quality database. If it is a new station, use streams names or features identifiable on topographic map if possible. However be specific enough so that someone else can find the sampling location.
   d. **County** is the county name where the station is located (even if most of drainage is in another county). For streams which form county boundaries, be consistent with other stations located on the stream.
   e. **WBID#/HUC:** When possible use Water Body ID Number (WBID#) assigned for the stream segment as assigned in the Assessment Database (ADB). Otherwise indicate 8 digit HUC code.
   f. **Latitude and Longitude** are to be recorded in decimal degrees measured by GPS. If it is a new site, the latitude and longitude will be recorded mid-stream in the middle of the sampling reach with a calibrated GPS. Always check latitude and longitude against the database for existing stations to verify location. If discrepancies are discovered, notify PAS of need to correct database information once you have confirmed you are in the correct location and GPS reading are accurate.
   g. **Assessors:** Include full name (initials may be used on subsequent pages). Include agency if not WPC.
   h. **Date:** Record date in mm/dd/yy format.
   i. **Time:** Indicate time of arrival at site in 24 hour clock (military time)

j. **Stream order**: Strahler order based on 24 scale topographic map or GIS layer. Do not use Gazetteer. This is necessary to know what biometrics ranges to use for scoring. (To save time >2 can be entered for larger streams).

k. **RM**: River mile should be indicated if not included in station ID (example reference streams).

l. **Drainage area**: Square mile drainage upstream of sampling location. Necessary in headwater streams (first or second order) to determine appropriate biorecon guidelines or biocriteria to use. Optional in larger streams. Drainage area can be quickly determined using the interactive map at http://water.usgs.gov/osw/streamstats/tennessee.html

m. **Watershed Group #**: Indicate watershed group based on cycle. This is especially important in watersheds split into 2 cycles such as 06060102, 06020001and 06010201.

n. **Ecoregion:** The ecoregion can be determined by locating the sample location at http://tnmap.tn.gov/wpc/ (make sure the ecoregion layer is checked). If the upstream drainage is in another ecoregion record this in U/S ECO. Contact PAS if uncertain of ecoregion.

o. **TOPO:** Optional, indicate topographic map where station is located.

p. **Gaz. Page**: Optional, indicate gazetteer page where station is located.

q. **Drainage Basin**: Optional indicate drainage basin where station is located.

r. **Project/Purpose:** Circle the appropriate reason for collecting sample or indicate reason in “other” slot.

2. Samples Collected.

Document EFO log number for each biological sample type collected. If chemical or bacteriological samples were collected at the same time, circle the appropriate parameters.

3. Field Measurements

Use calibrated meters for all field measurements (protocol C)

Designate what type of meter (and which meter) was used to make readings. The measurements for each parameter (including duplicates) are recorded in the appropriate boxes. All measurements are recorded in the units specified on the field sheet. Record any other field measurements, such as percent oxygen saturation, turbidity or TDS that were taken at the time of sampling (include units). Also record field measurements on the chemical request forms if chemical samples are being collected at the same time. (Average duplicate readings on the chemical request forms since only one value can be entered in the laboratory's system).

If, after the drift check, the meter was found to be off by more than 0.2 units for pH, temperature or dissolved oxygen (or more than 10% for conductivity or percent saturation), write an N before the measurement on the stream survey field sheet for all sites visited between the initial calibration and the drift check. The N designates questionable readings. Also, put an N before readings on the chemical request form. If the chemical request form has already been turned into the laboratory, fax the field sheet to the central office. This will insure the readings are flagged as questionable when they are entered into the water quality database.

Indicate any problems with the meters that would lead to questionable measurements. This line should be filled in anytime measurements are unusual even if it is only to verify that measurements are accurate.

Circle the appropriate level of precipitation for the previous 48 hours (or circle unknown). Also, indicate the ambient weather and record approximate air temperature.

4. Watershed Characteristics

Drive or walk as much of the upstream watershed as possible. Indicate the approximate percent of the watershed observed. Note the prevalent land-use in the watershed upstream of the sampling location in the spaces provided, estimating percentages. Record any other land uses that, although not dominant, may potentially affect water quality. These direct observations can be supplemented by land use information found in some of the recently developed TMDL/modeling tools and GIS systems. However, it should be noted that much of the land-use imagery is dated and ground-truthing is preferable when practical.

5. Physical Stream Characteristics

a. These observations apply to the land use surrounding the sampled stream reach. Indicate the length of the stream reach sampled in meters.

b. Observe and note the surrounding land use in the immediate stream reach for each bank. Use blank field to record any additional land use.

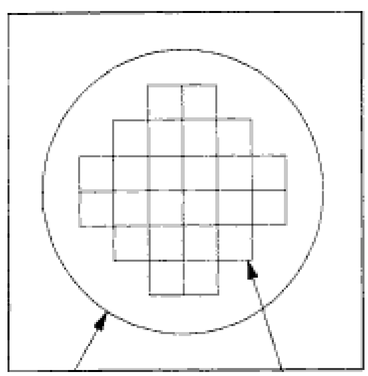
c. Indicate the magnitude of observed human disturbance to the stream. Describe any others not listed.

d. Canopy cover should be measured using a spherical densiometer mid-stream midway of the area(s) where biological samples were collected (mid-riffle if collecting one riffle, midway between two riffles if collecting multiple riffles, mid-distance between most upstream and most downstream bank sample if collecting bank jabs). The densiometer is a convex mirror etched into 24 ¼-inch boxes (Figure 8). Each box is subdivided into four smaller squares, via an imaginary dot in the center of the box, to create a total of 96 smaller squares that can be counted within the entire densiometer. Hold the densiometer one foot above the water surface. Holding the instrument at this level eliminates errors due to differing heights of samplers and different water depths, and includes low overhanging vegetation more consistently than holding the densiometer at waist level. Take four measurements, facing upstream, downstream, the right descending bank, and the left descending bank. Hold the instrument far enough away from the body so that the operator's head is just outside the grid. Count the number of small squares (out of a total of 96) that have tree canopy. Record this number (number of dots **WITH** canopy cover) on the field sheet. In order to get the overall percent canopy cover for that point, sum the four measurements and divide the total by 384 and multiply by 100%. Record this number in the measured field.

In addition to the measured canopy taken at mid sample point, estimate the canopy cover for the entire reach. Either circle the appropriate category or record a number in the estimated field.

Note: If also collecting periphyton, the densiometer measurements from the five transects recorded on the periphyton worksheet will take the place of these measurements.

Photo provided by Joellyn Brazille, Memphis EFO



Cook et al. 1995

Figure 8: Spherical Densiometer

e. Note any sediment deposits, type of sediment and water turbidity. Indicate the water color if not clear.

f. Note any substance that may be causing a surface sheen or foam including bacteria, nutrients or surfactants.

g. Record the estimated abundance and type of algae present. (Indicate if a periphyton survey was performed).

h. Estimate the average depth, width and length (in meters) of a typical riffle, run and pool within the survey reach. (Indicate if actual measurements are taken.)

i. Flow measurements are not required for biological surveys unless chemical samples were collected at the same time. However, if flow measurements were taken or if a staff gauge is available, record the calculated flow.

j. Record the high water mark and bank height in meters.

k. Circle the flow conditions, gradient and stream width that best describes the sample reach.

l. Estimate the percent contribution of each substrate type in riffles, runs and pools

6. <u>Field Based Assessment</u>

a. If biorecon was collected, record score. Indicate taxonomic level.

b. If SQSH was not collected and assessment is based on biorecon and field observations, indicate whether benthic community appears impaired

c. Describe basis for determination if based on biorecon. If supporting, indicate observations that led to this conclusion. If impaired, describe observations that led to this conclusion, for example low abundance of EPT. Include observed impacts and possible sources.

d. Describe any other conditions observed at the time of sampling (additional pages are to be used if necessary). Include any changes observed from previous sampling efforts. Note directions to the site and any special permission or keys needed for access. Note anything special or unusual that would assist in assessments. Ask other team members for input. Take care not to contradict information provided on other parts of the sheet or on the habitat field sheet, for example sedimentation, erosion and algae observations.

e. A photographic record is to be kept on each sampling station. Digital photographs are preferred. Photographs of the general stream condition and potential pollution sources should be taken during the original sample visit. Photographs of any changes are taken during subsequent sampling trips. Document the picture identification and a brief description on the field sheet.

f. Stream Sketch - A station sketch is made at the time of sampling. This sketch should be detailed enough so that subsequent sampling teams or data reviewers can determine where samples were taken and what potential sources of impairment were present. Use a separate sheet of paper if necessary. At a minimum, the sketch should include a rough outline of stream sinuosity, direction of flow, location of riffles and pools, location of samples (benthic, chemical, field parameters), location of bridges or any other man-made structures (include distance from sampling point), location of tributaries, run-off ditches, discharges, livestock access, and any other potential pollution sources. It is helpful to designate which direction is north.

Note: A small table is provided in this section for those who wish to transcribe biorecon and habitat information from other field sheets. This information is not required since it is included elsewhere. If using this feature, make sure that information matches both the biorecon and habitat field sheets and that any later changes or corrections are made on all sheets.

Protocol F - Biorecon (Reconnaissance/Screening)

Biologist/Environmental Specialist with expertise in macroinvertebrate taxonomy
Because the biorecon is qualitative and involves limited data generation, its effectiveness depends largely on the experience of the biologist performing the assessment. The biologist should have stream assessment experience, knowledge of aquatic ecology and expertise in benthic macroinvertebrate taxonomy and community structure for the ecoregion in which they are conducting the assessment.

The Biorecon method is based on EPA's *Rapid Bioassessment for Use in Wadeable Streams and Rivers* (Barbour et. al., 1999).

This method is a standardized screening tool useful for problem identification and/or prioritizing sites for further assessment, monitoring or protection. As specified in the EPA document, it is designed to be expedient and requires an experienced and well trained biologist to be effective. It is most useful in discriminating clearly impaired or non-impaired areas from those areas requiring further investigation. However, chemical samples, in-stream water quality measurements, field observations, professional expertise, estimates of taxa abundance, identification to a lower taxonomic level and/or habitat data can help clarify assessment decisions in ambiguous situations. Biorecons cannot be compared to biocriteria or to semi-quantitative samples. Only qualitative metrics which do not include relative abundance can be calculated. However, metrics may be compared to the biorecon guidance derived from biorecons conducted at ecoregion reference sites (Tables 4 and 5).

The flow charts in Protocol A should be used when determining when biorecon sampling is appropriate.

1. <u>Biorecon Field Sheet Header</u>

   Complete all header information on biorecon field sheet (Appendix B). See protocol E for completion of Station ID, Stream Name, Station Location, HUC, Group, Assessors, Date, Time, Ecoregion, U/S ecoregion, Order, Drainage Area. See protocol H for assigning log number. (A log number must be assigned even if a voucher is not collected for database entry and tracking).

   If this is a QA/QC sample, circle the appropriate type(s): Duplicate sample, pick efficiency, ID verification (note that ID verification is confirmation of identification by a second taxonomist, not the initial identification of voucher specimens). Include date and initials of taxonomist for any vouchers that have been identified in the space provided.

2. <u>Taxonomic Level</u>

   Unless this is an ecoregion or headwater reference site, either genus level or family level biorecons can be conducted. (Both genus and family must be reported at reference sites).

Genus level biorecons are more sensitive but require more time and taxonomic expertise. Often family level biorecons are adequate screening tools. If more sensitivity is needed, a semi-quantitative sample may be more useful than a genus level biorecon. It is important that taxonomic level is consistent. For example all taxa (except chironomids, oligochaetes, acarina, nematodes, and nematomorphs) should be identified to genus and the genus guidelines used or all taxa should be identified to family and the family guidelines used.

3. Habitat Selection

Determine what habitat is available and the relative percent contribution of each habitat. Record percent habitat on the biorecon field sheet (even for those not sampled).

Select 1 to 4 of the most productive habitats for sampling. Only consider habitats that comprise more than 5% of the stable habitat in the stream -reach. Productive habitats include riffles, run or pool rocks, leaf packs, woody debris, rooted undercut banks, macrophyte beds, and fine sediment. Other productive habitats that comprise more than 5% of the stream reach may also be selected. Estimate approximately 0.5 meters of sampling area for any habitat selected.

Proportion the selected habitats into four portions based on percent contribution. For example, if the selected habitats are riffle (50%), leaf packs (30%) and undercut banks (20%) the sample would be comprised of two riffle kicks, one leaf jab and one bank jab. Never collect more than four portions. Record the the number of jabs collected in each habitat on the Biorecon Field Sheet.

4. Sample Collection

Sample selected habitat*s* using a 500 micron mesh triangular or dip net. Use a combination of kick and/or jab techniques depending on the type of habitat. A single jab consists of thrusting the net into a productive habitat for a linear distance of 0.5 meter (approximately two net widths). A kick is a stationary sample accomplished by positioning the net and disturbing the upstream substrate for a distance of 0.5 meter. Take care not to over-sample since this could skew results as reference data is calibrated to 4 jabs from a maximum of 4 habitats.

a. Riffle Kick:

Position the net on the bottom of the stream and disturb the substrate by shuffling and kicking your feet the width of the net and for approximately 0.5 meter upstream of the net. Use hands or soft brush to scrape clinging organisms off larger rocks.

b. Run or Pool Rock:

Select several rocks of various sizes equaling approximately 0.5 square meters of surface area in run or pool areas. (Note these are separate habitats.) Avoid rocks that are embedded.

c. Leaf Packs:

Collect three handfuls of leaf packs by positioning the net downstream or under the leaf pack, then scooping the leaves into the net by hand. Select leaf packs from various locations (riffle, run, and pool if possible). The leaves should be submerged and show evidence of being consumed by benthic macroinvertebrates (50% decomposition is optimal). Avoid collecting recently deposited or fully decomposed leaf litter.

d. Snags/Woody debris:

Select snags and other woody debris that have been submerged for a relatively long period (not recent deadfall). Sample submerged woody debris by jabbing in medium-sized snag material (sticks and branches). The snag habitat may be kicked first to help dislodge organisms, after placing the net downstream of the snag. Accumulated woody material in pool areas are also considered snag habitat. A single jab is approximately two net widths or three handfuls of loose material.

e. Undercut Banks or Tree Roots

Select bank habitat that is undercut with submerged hanging roots or plants. Submerged tree roots that are not undercut may also be sampled (this constitutes a different habitat type). Thrust the net vigorously under the bank to dislodge clinging organisms. A single jab is approximately two net widths. Avoid digging into the sediment, as this constitutes another habitat type.

f. Macrophytes

In deep water, sample aquatic plants that are rooted on the bottom of the stream by drawing the net through the vegetation from the bottom to the surface of the water. In shallow water, bump or jab the net along the bottom in the rooted area. Avoid collecting sediment if possible. Either type of macrophyte jab should not exceed 0.5 linear meters (two net widths).

g. Fine sediment

Sediment is found in quiet areas of the stream. Select fine silt, sand or muck with minimal gravel. Seek areas with evidence of tunneling by burrowing macroinvertebrates. Gently scoop the net through the sediment for 0.5 meters length and approximately 4 cm deep.

5. Sample Sorting/Identification

Composite the four jabs in a 500-micron sieve (or the net). Examine large materials (rocks, leaves, sticks) for organisms and then discard. Rinse the remainder of the debris using sieved water in the sieve (or swish the net in the creek). Transfer small amounts of debris to a white pan with a little sieved stream water for field sorting. Keep the rest of the sample submerged. Scan the debris and water for organisms. If doing a **family biorecon**, record field identification (or description for later identification at field office if uncertain.) Unless this is a QC site or identification is uncertain, vouchers do not need to be routinely collected for family biorecons. Vouchers of any families where ID is uncertain and complete vouchers of all taxa at 10% of sites must be collected. Vouchers should also be collected if there is a possibility that more definitive (genus level) identification may be necessary.

If it is a genus level biorecon, record field identification or description of each distinct taxon on the biorecon field sheet (so that you can recognize it back at the office). Voucher specimens of each unique taxon are required for genus level identifications. Any taxa not found on the Tennessee taxa list (Appendix C) must be sent to the lab for expert verification and inclusion in state reference collection.

On the biorecon field sheet for both family and genus biorecons, record the relative abundance of each taxon (rare, common, abundant, dominant). This will help with determination of impairment in ambiguous biorecons. Indicate relative abundance for both field identification and voucher identification (f collected) in the appropriate boxes for each taxon on the biorecon field sheet. If, after voucher identification, relative abundance cannot be estimated (for example multiple taxa were identified that were not differentiated in the field), place an x in the voucher box for that taxon.

1 = rare (1-3 organisms)
2 = common (4-9 organisms)
3 = abundant (10-49 organisms)
4 = dominate (> 50 organisms)
x = uncertain (used to indicate voucher identification with uncertain abundance)

6. Voucher Specimens

a. Family level biorecons. Vouchers (of all taxa in sample) are only required at 10% of sites and any taxon where family cannot be field differentiated (for example Leuctridae/Capniidae; Leptohyphidae/Caenidae; Polymitarcyidae/Ephemeridae; Baetidae/Ameletidae; Corduliidae/Libellulidae; Psychomyiidae/Polycentropodidae; Unenoidae/Limnephilidae/Odontoceridae; or early instars).

b. Genus level biorecons. Vouchers are required at all sites. Taxa that are not included on Tennessee taxa list (Appendix C) must be sent to the lab for expert verification and inclusion in state reference collection.

Place one or more representatives of each taxon in a small bottle containing 80% ethanol. If identifying to genus level, preserve several individuals of families that commonly have multiple genera that are difficult to differentiate in the field. This includes Heptageniidae, Hydropsychidae, and many stonefly families. Include individuals that vary in color and/or size. Since the biorecon is designed to be a fast screening tool, try to limit voucher specimens to a few representatives that are thought to be distinct taxa. This will be easier with experience. If more definitive identifications are necessary, it would probably be more appropriate to collect a semi-quantitative sample. Place an internal tag with the station ID, date, sampler's initials and sample type inside the bottle (Figure 9). Attach an external sample tag to the outside of the bottle (Figure 10). Alternatively, the same information can be written on the outside of the bottle using indelible ink.

DAVIS0012.5CL COL: JEB/DRM 3/6/02 BIORECON

Figure 9: Example of internal tag.

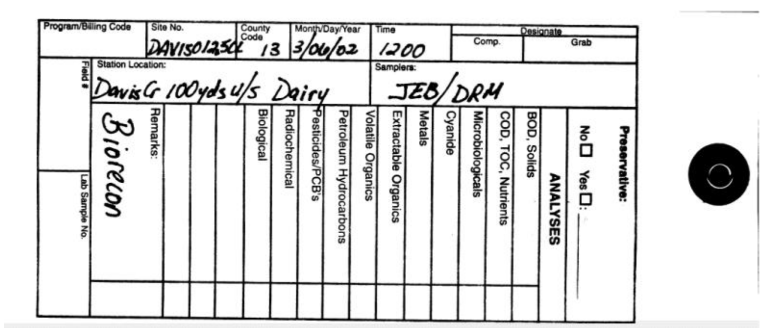


Figure 10: External tag

At the office, check the voucher organisms using a dissecting scope for accuracy in field identification. When conducting genus level biorecons identify all taxa to genus except chironomids, oligochaetes, acarina, nematodes, and nematomorphs. Whichever taxonomic level is selected, be consistent throughout the study. For example, do not identify EPT to genus if other organisms are only identified to family. Record appropriate changes for field misidentifications on the biorecon field sheet and indicate relative abundance (or x if uncertain) for each verified taxon. Retain voucher animals for five years for QC purposes or in case further identification is needed at a later date. Add the name of the taxonomist, log number and date identified to the existing internal and external field tags (Figure 11).

DAVIS0012.5CL
COL: JEB 3/6/02
BIORECON
ID: JEB 3/7/02
K0203001

Figure 11: Example of internal tag after sample identification

Any genera identified, that are not on the verified taxa list in Appendix E should be sent to the TDH lab for verification and inclusion in the statewide reference collection.

7. Biometric Calculation – After confirming field identifications, calculate three qualitative biometrics (that do not rely on relative abundance). Do not calculate NCBI or any other metric that is not qualitative. Never include exuvia, empty caddis cases or empty mollusk shells when calculating biometrics although these can be noted for additional information.

Record metric values, score and total score for either family or genus (or both if reference site) on the biorecon field sheet.

a. **Taxa Richness (TR)** – The total number of distinct taxa found at a site. Do not include empty caddis cases, empty mollusk shells, exuvia, non-aquatics or winged adults. Do not count unidentified genera as a separate taxon, unless they are clearly a different genus (or family) than those identified. When counting chironomid taxa for family level biorecons, all chironomids are counted as a single family, when counting chironomids at genus level, red midges, non-red midges and tanypodinae are counted separately for a maximum total of three genera. Chironomids should be categorized in the field and there is no need to collect vouchers since they will lose color. Otherwise Chironomini will be counted as red midges. Orthocladinae, Diamesinae and Tanytarsini will be counted as non-red midges.

b. **EPT** – The total number of distinct EPT taxa found at the site. (Do not include empty caddis cases, exuvia, non-aquatics or winged adults). Do not count unidentified genera as a separate taxon, unless they are clearly a different genus (or family) than those identified.

c. **Intolerant Taxa (IT)** – The number of intolerant taxa (defined as having an NCBI value from 0.00 to 3.00) found at the site. Appendix C contains a list of families that should be counted as intolerant. For statewide consistency and comparability to the reference database, DO NOT count any animals intolerant that are not on this list.

When doing genus level biorecons, if an animal can only be identified to family and there are no other genera present in that family, it should be considered intolerant if it is an intolerant family. Likewise, for new genera where a tolerance value has not been assigned, it will be considered an intolerant taxa if the family is tolerant (see protocol k for assigning NCBI values).

NOTE: Although the biorecon procedure calls for relative abundance to be estimated in the field, these numbers cannot be used for any metric calculations since they are not collected or subsampled in a quantitative manner. This information can be used less formally to evaluate the health of the macroinvertebrate community especially in the case of ambiguous scores. For example, if the number of EPT taxa is high, but only a few individuals were found, it may be indicative of stress in the community.

8. Assessment Guidelines – Determine the value (1, 3, or 5) for each metric based on ranges at either the family (Table 3) and/or genus (Table 4) level. Add the three values together. Determine the assessment based on the total score (page 10). Do not use the family score if organisms were identified to genus level. The genus level provides a more accurate assessment and may not agree with the family level guidance. Keep the following information in mind when assessing biorecon data:

   a. Biorecons are most useful in areas of clear impairment or in areas that are not impaired. Sites that fall in the middle range (7 – 9) may be too ambiguous to make assessments using the biorecon technique. These sites may require a more intensive assessment method. A Semi-Quantitative Single Habitat Sample (SQKICK or SQBANK) can be collected for clarification of use support (Protocol G). Chemical samples, in-stream water quality measurements, field observations, professional judgment and habitat data may also help clarify assessment decisions.

   b. Preliminary guidelines have been developed for headwater (< 2 sq mile) drainage in some bioregions. In other bioregions an upstream, watershed or ecoregional reference must be collected at the same time for comparison to test sites.

   c. Comparisons to the ecoregion reference guidelines are not appropriate at sites whose upstream watershed has drainage of more than 20% in another bioregion. Data should be scored using both bioregions. If scores disagree, professional judgment should be used to make assessment.

Table 3: Assessment Guidance for Family Level Biorecons.

Bio-region	Season	Drainage area * (sq miles)	Taxa Richness (TR)			EPT			Intolerant Taxa (IT)		
Score			**5**	**3**	**1**	**5**	**3**	**1**	**5**	**3**	**1**
65abei-74b	Jan-Dec	> 2	> 16	9-16	< 9	> 6	3-6	< 3	> 2	1-2	< 1
65abei	Jan-Dec	≤ 2	> 15	8-15	< 8	> 7	4-7	< 4	> 4	3-4	< 3
74b	Jan-Dec	≤ 2	> 12	6-12	< 6	> 3	2-3	< 2	> 1	1	< 1
65j	Jan-Dec	All	> 18	10-18	< 10	> 8	4-8	< 4	> 5	3-5	< 3
66deik	Jan-Dec	All	> 20	10-20	<10	>12	6-12	< 6	> 8	4-8	< 4
66fgj	Jan-Dec	All	> 23	12-23	< 12	> 12	7-12	< 7	> 9	5-9	< 5
67fghi	Jan-Dec	> 2	> 20	10-20	< 10	> 8	5-8	< 5	> 5	3-5	< 3
67fghi	Jan-Dec	≤ 2	> 19	10-19	< 10	> 10	5-10	< 5	> 6	4-6	< 4
68a	Jan-June	All	> 20	10-20	< 10	> 9	5-9	< 5	> 5	3-5	< 3
68a	July-Dec	All	> 22	11-22	< 11	> 10	6-10	< 6	> 6	4-6	< 4
68b	Jan-Dec	> 2	> 13	7-13	< 7	> 5	3-5	< 3	> 3	2-3	< 2
68b	Jan-Dec	≤ 2	> 13	7-13	< 7	> 2	2	< 2	> 1	1	< 1
68cd	Jan-June	All	> 12	7-12	< 7	> 7	4-7	< 4	> 4	2-4	< 2
68c	July-Dec	All	> 15	8-15	< 8	> 8	4-8	< 4	> 5	3-5	< 3
69de	Jan-June	All	> 20	10-20	< 10	> 10	6-10	< 6	> 8	4-8	< 4
69de	July-Dec	All	> 18	10-18	< 10	> 8	4-8	< 4	> 6	3-6	< 3
71e	Jan-Dec	All	> 18	10-18	< 10	> 7	4-7	< 4	> 4	3-4	< 3
71fgh	Jan-Dec	> 2	> 19	10-19	< 10	> 9	5-9	< 5	> 6	3-6	< 3
71fgh	Jan-Dec	≤ 2	> 17	9-17	< 9	> 8	5-8	< 5	> 6	3-6	< 3
71i	Jan-Dec	> 2	> 19	10-19	< 10	> 6	4-6	< 4	> 4	2-4	< 2
71i	Jan-Dec	≤ 2	> 12	7-12	< 7	> 4	3-4	< 3	> 2	2	< 2
73ab**	Jan-Dec	> 2	> 13	7-13	< 7	> 1	1	<1	NA	NA	NA
73ab**	Jan-Dec	≤ 2	NA	NA	NA	NA	NA	NA	NA	NA	NA
74a	Jan-June	> 2	> 11	6-11	< 6	> 3	2-3	< 2	> 1	1	< 1
74a	July-Dec	> 2	> 12	7-12	< 7	> 3	2-3	< 2	> 1	1	< 1
74a	Jan-Dec	≤ 2	NA	NA	NA	NA	NA	NA	NA	NA	NA

* Caution should be used in streams ≤ 2 square mile drainage

** It is recommended that biorecons not be used in ecoregion 73 since the intolerant richness metric is not applicable.

Table 4: Assessment Guidance for Genus Level Biorecons.

Bio-region	Season	Drainage area * (sq miles)	Taxa Richness (TR)			EPT			Intolerant Taxa (IT)		
Score			**5**	**3**	**1**	**5**	**3**	**1**	**5**	**3**	**1**
65abei-74b	Jan-Dec	> 2	> 20	10-20	< 10	> 7	4-7	< 4	> 2	1-2	< 1
65abei	Jan-Dec	≤ 2	> 17	9-17	< 9	> 7	4-7	< 4	> 4	3-4	< 3
74b	Jan-Dec	≤ 2	> 14	8-14	< 8	> 3	2-3	< 2	> 1	1	< 1
65j	Jan-Dec	All	> 22	12-22	< 12	> 11	6-11	< 6	> 6	4-6	< 4
66deik	Jan-Dec	All	> 30	15-30	<15	>18	10-18	< 10	>16	8-16	< 8
66fgj	Jan-Dec	All	> 34	18-34	< 18	> 22	11-22	< 11	> 17	9-17	< 9
67fghi	Jan-Dec	> 2	> 31	16-31	< 16	> 13	7-13	< 7	> 9	5-9	< 5
67fghi	Jan-Dec	≤ 2	> 28	14-28	< 14	> 14	8-14	< 8	> 10	6-10	< 6
68a	Jan-June	All	> 32	17-32	< 17	> 18	10-18	< 10	> 11	6-11	< 6
68a	July-Dec	All	> 36	18-36	< 18	> 17	9-17	< 9	> 11	6-11	< 6
68b	Jan-Dec	> 2	> 15	8-15	< 8	> 7	4-7	< 4	> 3	2-3	< 2
68b	Jan-Dec	≤ 2	> 13	7-13	< 7	> 3	2-3	< 2	> 1	1	< 1
68cd	Jan-June	All	> 14	7-14	< 7	> 7	4-7	< 4	> 6	4-6	< 4
68c	July-Dec	All	> 15	8-15	< 8	> 6	3-6	< 3	> 5	3-5	< 3
69de	Jan-June	All	> 29	15-29	< 15	> 17	9-17	< 9	> 12	6-12	< 6
69de	July-Dec	All	> 26	14-26	< 14	> 13	7-13	< 7	> 8	4-8	< 4
71e	Jan-Dec	All	> 22	12-22	< 12	> 9	5-9	< 5	> 6	3-6	< 3
71fgh	Jan-Dec	> 2	> 25	13-25	< 13	> 12	7-12	< 7	> 8	5-8	< 5
71fgh	Jan-Dec	≤ 2	> 22	11-22	< 11	> 11	6-11	< 6	> 8	4-8	< 4
71i	Jan-Dec	> 2	> 23	12-23	< 12	> 6	4-6	< 4	> 3	2-3	< 2
71i	Jan-Dec	≤ 2	> 14	8-14	< 8	> 5	4-5	< 4	> 3	2-3	< 2
73ab**	Jan-Dec	> 2	> 10	6-10	< 6	> 1	1	<1	NA	NA	NA
73ab**	Jan-Dec	≤ 2	NA	NA	NA	NA	NA	NA	NA	NA	NA
74a	Jan-June	> 2	> 14	7-14	< 7	> 3	2-3	< 2	> 1	1	< 1
74a	July-Dec	> 2	> 14	8-14	< 8	> 4	2-4	< 2	> 1	1	< 1
74a	Jan-Dec	≤ 2	NA	NA	NA	NA	NA	NA	NA	NA	NA

* Caution should be used in streams ≤ 2 square mile drainage

** It is recommended that biorecons not be used in ecoregion 73 since the intolerant richness metric is not applicable.

Score Interpretation for family and genus level biorecons:

For all bioregions except 73ab:
11-15 = Non-impaired (Supporting)
6-10 = Ambiguous (Need Additional Data)
≤ 5 = Severely Impaired (Partially or Not-Supporting)

For bioregion 73ab:
7-10 = Non-impaired (Supporting)
3-6 = Ambiguous (Need Additional Data)
≤ 2 = Severely Impaired (Partially or Not Supporting)

Scoring for headwater streams for ecoregions not included in Tables 3 or 4:

In headwater streams where an ecoregion reference score has not been calculated, it will be necessary to sample a reference site at the same time. A reference will also need to be collected in atypical streams that are not directly comparable to reference guidelines. Ideally this will be an ecoregion reference. Otherwise an upstream or watershed reference should be used. In order to compare sites trisect the reference value for each metric (taxa richness, EPT richness and intolerant taxa):

Score 5 > One less than [metric value – (metric value/3)]
Score 3 < lowest score 5 – (metric value/3) to one less than lowest score 5
Score 1 < lowest score 3

For example, in ecoregion 68b insufficient samples have been collected to calculate headwater reference guidelines. So, if an assessment needed to be conducted on a reference stream in 68b using a biorecon, a reference sample would be collected at the same time.

To calculate the reference-based scoring for family richness, if the number of distinct families at the reference site were 20:

Score 5 would be ≥ 20 – (20/3) which is 13

Since 13 distinct families is the lowest number that would score a 5, in order to calculate the range for a score of 3 would be < 13 – (13/3) to 13 (or 9 to 12)

Since 9 is the lowest number of families that would score a 3, in order to calculate a range for a score of 1 would be < 9

The same procedure would be used to calculate the scoring ranges for EPT richness and intolerant richness or for genus level.

Protocol G – Field Collection Techniques for Semi-Quantitative Single Habitat Sample (SQKICK or SQBANK)

Biologist or Environmental Specialist

Collect a semi-quantitative single habitat sample (SQKICK or SQBANK) when a quantifiable assessment of the benthic community is needed. **See flow charts Protocol A for guidance.** This method is directly comparable to the Division's numeric translators for biocriteria. This is a more defensible and sensitive method than the biorecon. When both sample types have been collected, semi-quantitative sample results will take precedence over biorecon results.

The semi-quantitative single habitat sample will generally be used for:

a. 303(d) list removal or addition (a biorecon can be used if it shows the site clearly supporting or non-supporting)
b. Nutrient TMDLs
c. Permit compliance and enforcement
d. Exceptional Tennessee Water designation for exceptional biological diversity.
e. Pre/post BMP or ARAP
f. CADDIS analysis
g. Trend Analysis
h. Ecoregion or headwater reference sites (along with genus level biorecon)
i. Confirmation of ambiguous biorecons.
j. Any study that has the potential of being used by the Water Quality Control Board.

In order for the data to be compared to the reference database:

a. Samples must be collected in the exact manner outlined in this section.
b. The upstream watershed must be 80% within the bioregion.
c. The stream size must be comparable to those in the reference database for that bioregion (Appendix A).

There are three methods of semi-quantitative sample collection:

a. SQKICK (Riffle streams larger than 1 meter wide)
b. Modified SQKICK (Riffle streams less than 1 meter wide or too shallow for the 1 meter kick net)
c. SQBANK (Non-riffle streams)

The type of sample collected will depend on the stream type and/or ecological subregion. Ecoregions can be determined for specific stream segments by using Tennessee's Online Water Quality Assessment Database (http://tnmap.tn.gov/wpc/). Contact the Planning and Standards section if there is uncertainty about what ecoregion a stream is located in.

a. **Semi-quantitative Riffle Kick (SQKICK)**

Collect a semi-quantitative riffle kick (SQKICK) in ecological subregions 65j, 66d, 66e, 66f, 66g, 67f, 67g, 67h, 67i, 68a, 68b, 68c, 69d, 71e, 71f, 71g, 71h, 74a, and riffle streams in 71i. If a riffle is not present, a semi-quantitative bank sample can be collected, but will not be directly comparable to the reference criteria. Therefore, an upstream or off-site reference SQBANK will also need to be collected (this can be a bank sample collected at one or more of the established ecoregion reference sites). If riffles have been compromised by sedimentation or are embedded, they should still be sampled since impacts are being measured. For small (< 1 meter wide) or shallow streams, the modified kick method (protocol, see b, should be used).

1. Use a (two-person) one square meter kick net with a 500-micron mesh to sample the riffle. If necessary, use rocks to weight the bottom edge to prevent the flow of water beneath the net. At each site, collect two kicks: one from an area of fast current velocity and one from an area of slower current velocity. Always collect the downstream sample first. Avoid areas with large leaf packs caught on the rocks if possible. If the stream is too small to do two riffle kicks in a single riffle, sample two separate riffles. (In extremely small or shallow streams, sample 4 riffles using the modified SQKICK for small streams – method b.)

2. One biologist holds the net at an angle that allows the current to flow into it making sure the bottom is in contact with the substrate and the top of the net is above the surface of the water. The net should have the maximum wetted area by laying it back as far as possible, while keeping the top of the net above the surface of the water. The second biologist disturbs the substrate for approximately one-meter distance and the width of the net (one meter) upstream of the net by kicking and shuffling the substrate. This causes organisms and debris to flow into the net. Larger rocks may be lifted and rubbed with the hands or a soft brush to remove clinging organisms.

3. Once the kick is completed, allow time for the lighter debris to finish floating into the net. The biologist who performed the kick then grabs the two pole ends at the bottom of the net and carefully lifts the net out of the water while the other biologist continues to hold the upper end making sure the top of the net does not dip below the water surface allowing organisms to escape. If the top of the net dips under the water and debris flows out, discard the sample and collect another kick. Carry the net horizontally to the bank for processing.

4. Composite the debris from both kicks. Carefully position the net in a 500 micron sieve bucket. Rinse debris and organisms, with sieved water, off the net into the sieve bucket. Make sure to get all debris and organisms. Thoroughly rinse the sample using sieved water to remove fine sediment. Large rocks or organic material, such as whole leaves or twigs, are discarded after rinsing and removing clinging organisms. Transfer the sample to a wide mouth or plastic container. Using forceps, remove all organisms clinging to the net

and add them to the sample container. If upon cursory examination of the debris, it does not appear that a minimum of 200 organisms have been collected after 2 kicks, perform additional kicks in the same reach until at least 200 organisms are assured. Document the number and location of kicks on the stream survey field sheet and write the number of kicks on the sample tag. If all riffle habitat is exhausted before 200 organisms are collected, document on the stream survey field sheet. Note that samples where more than 2 kicks are necessary to find 200 organisms will be evaluated with extra caution regardless of score.

5. Preserve the sample with 80% ethanol. Note that if a large amount of debris is present in the sample, 90% or stronger ethanol should be used. Include an internal tag (written in pencil on water-proof paper) with the station ID, date, sampler's initials and sample type inside the container with the debris (Figure 7). Attach an external sample tag to the outside of the container. Standard external tags for both biological and chemical samples are obtained from the state lab (Figure 8). Instead of an external tag, the site information can be printed in indelible ink (i.e. Sharpie) on the sample lid. The external tag information must include the Station ID, Stream name, location, sampler's initials, date sampled, time sampled and sample type (Figure 12). A biological sample request form, including chain of custody, must be completed prior to delivery to the state lab for identification (Appendix B).

Figure 12: Example of external tag information (on sample lid)

b. Modified SQKICK (small and/or shallow streams)

1. In extremely small streams, where riffles are less than one meter wide, or in those that are too shallow to use a 1 meter kick net, collect a one person stationary kick using an 18-inch single handle rectangular net with a 500-micron mesh.

2. Sample four separate riffles. Starting with a downstream riffle, hold the net perpendicular to the flow making certain the bottom of the net is in contact with the substrate at all times. Disturb the substrate upstream of the net for an area approximately 18 inches long and the width of the net. Do not allow the net to move during the kick as it might cause organisms to drift under the net. Once the kick is complete, allow time for all debris to finish flowing into the net.

3. Composite the debris from all four kicks. Use forceps, to remove all organisms clinging to the net and add them to the debris. Thoroughly rinse the sample using sieved water to remove fine sediment. Large rocks or organic material such as whole leaves or twigs, are discarded after rinsing and removing clinging organisms. If upon cursory examination of the debris, it does not appear that 200 organisms are in the composited sample, collect additional kicks and add them to the composite. If all riffle habitat is exhausted before 200 organisms are collected, document on the stream survey field sheet. Document the total number of kicks on the sample tag and on the stream survey field sheet.

4. Place the composited debris in a wide mouth plastic container and preserve with 80% ethanol (use a stronger concentration if there is a lot of debris in the sample). Include an internal tag with the station ID, date, sampler's initials and sample type inside the container with the debris (Figure 7). Attach an external sample tag to the outside of the container. Standard external tags for both biological and chemical samples are obtained from the state lab (Figure 8). Instead of an external tag, the site information can be written in indelible ink (i.e. Sharpie) on the sample lid. The external tag information must include the Station ID, Stream name, location, sampler's initials, date sampled, time sampled and sample type (Figure 10). A biological sample request form including, chain of custody. must be completed prior to delivery to the state lab for identification (Appendix B).

c. Semi-Quantitative Bank Sample (SQBANK)

In ecoregions 65a, 65b, 65e, 65i, 73a, 74b, collect a semi-quantitative bank sample (SQBANK) (even if riffles are present) for comparison to the reference criteria. Also, use the SQBANK method in 71i streams without riffle areas.

1. Use a triangular dip net with a 500-micron mesh to sample the rooted undercut bank. Collect the samples by jabbing the net below the surface of the water using an upward/forward thrusting motion designed to dislodge macroinvertebrates from the roots. Sample three separate areas of the reach including at least one sample from each bank if possible. Collect samples from different velocities and different bank types (i.e. overhanging tree roots, undercut grass banks) if possible. Macrophyte beds may be substituted if rooted banks are not available. Sample approximately one linear meter (approximately 3 triangular net widths) at each of the three sampling locations. It may be necessary to collect more than 3 sampling location to get 9 linear meters if habitat areas are small.

2. Thoroughly rinse the sample by gently swishing the net through the water. Do not let the net opening dip below the surface of the water. Visually inspect any large organic matter such as whole leaves and sticks. Remove any organisms clinging to these materials and add to the smaller debris, before discarding the large material. Using forceps, remove any organisms clinging to the net and add to the sample. Composite the debris from all three bank samples. If upon cursory examination of the debris, it does not appear that 200 organisms have been collected, additional bank samples may be collected. If all rooted bank habitat is exhausted before 200 organisms are collected, document on the stream survey field sheet. Document the total number and location of bank jabs on the stream survey field sheet and the number of bank jabs on the sample tag.

3. Place the composited debris in a wide mouth plastic container and preserve with 80% ethanol (a stronger concentration should be used if there is a lot of organic matter in the sample). Include an internal tag with the station ID, date, sampler's initials and sample type inside the container with the debris (Figure 7). Attach an external sample tag to the outside of the container. Standard external tags for both biological and chemical samples are obtained from the state lab (Figure 8. Instead of an external tag, the site information can be written in indelible ink (i.e. Sharpie) on the sample lid. The external tag information must include the Station ID, Stream name, location, sampler's initials, date sampled, time sampled and sample type (Figure 10). A biological sample request form, including chain of custody, must be completed prior to delivery to the state lab for identification (Appendix B).

Protocol H: Sample Logging and Lab Transport

Any staff member

Samples must be logged to allow complete reconstruction, from initial field records, through data storage and system retrieval. This includes biorecons that are identified in the field with no vouchers. Assign a discrete log number to each individual macroinvertebrate sample (including biorecons). For field offices, this will be an eight digit number determined in the following manner:

N0105001

Where:

The first digit (N) determines which office the sample is from:

C = Chattanooga EFO	K = Knoxville EFO
L = Columbia EFO	M = Memphis EFO
V = Cookeville EFO	N = Nashville EFO
H = Johnson City EFO	S = Surface Mining
J = Jackson EFO	

The second and third digit represent the year sampled (01 = 2001)

The fourth and fifth digit represent the month sampled (05 = May)

The last three digits represent a consecutive number for the number of samples collected that month (001 = the first sample collected in May).

The field log numbers (along with the station ID) will be used to identify the sample on paperwork, tags, bench field sheets, logbooks, QC records, or any other place this sample is documented. Note for samples sent to the TDH lab for analysis, a second log number will be assigned by their LIMS system. Both the field number assigned by the collector and the LIMS number will follow the sample through analysis and be reported on data sheets.

A log is to be kept in the field office of all biological samples collected (Figure 13). It is recommended that this be an electronic log. A back-up copy in a separate location must be kept of all logs. The log entry must include the log number, station ID, date collected, time collected, collector’s initials, sample source, station location, type of sample and date sent to the lab.

A second log will be kept at the lab which will also include sample identification information (Figure 14).

<u>Transport to Lab</u>

All semi-quantitative samples collected by WPC environmental field offices are to be sent to the state laboratory (TDH) for identification as soon as possible after collection. (Samples collected by non-WPC staff may be identified by any qualified macroinvertebrate taxonomist who has met quality assurance requirements specified in Section II and follows the subsampling and taxonomic protocols specified in this document). The Nashville Lab is the only state laboratory with the capability to identify macroinvertebrate samples. The Knoxville and Jackson labs are certified to ship samples containing ethanol so samples may be delivered to any of the three labs Ethanol is considered by OSHA to be a hazardous material, only certified personnel may ship samples. Any certified WPC staff can also ship samples directly from the EFO.

A biological sample request form, including chain of custody must be completed and accompany all biological samples (Appendix B). The Aquatic Biology Section no longer receives samples directly. All samples are to be delivered to the lab's sample coordinator for processing.

Sample priorities:

Biological sample priorities are set by the Planning and Standards Section who coordinates with the state lab. Before completing the "date needed" on the sample request form, contact PAS if results are needed outside the following priority.

a. Watershed sample (including TMDLs and ecoregion sites) completed by December 31 of the fiscal year following the monitoring year for the group provided no sample is completed more than one year after receipt. For example Group 5 sample collection will start in July 2010 and end June 2011. Sample analysis is to be completed no later than December 2011. EFO QC samples will be completed the same time as the watershed group due date.

b. Anti-degradation samples within 30 days of receipt.

c. Special projects by agreed upon date as stipulated by grant.

d. Priority samples – contact Aquatic Biology Section, if lab receives too many priority requests, PAS will coordinate.

Log #	Station ID	Source	Location	Date Col.	Time Col.	Init. Col.	Type	Date sent to lab
K0203001	DAVIS012.5CL	Davis Ck	100 yds u/s Dairy	3/6/02	1200	JEB/DRM	SQKICK	9/10/10
K0203002	DAVIS001.3CL	Davis Ck	Hwy Z	3/6/02	1500	JEB/DRM	BR	NA

Figure 13: Macroinvertebrate Sample Collection Log

Lab Log #	Field Log #	Station ID	Source	Location	Date Col.	Time Col.	EFO	Init. Col.	Type	Date Received by Lab	Sort By	Sort Date	ID By	ID Date	Date Reported
N000053210	K0203001	DAVIS012.5 CL	Davis Ck	100 yds u/s Dairy	3/6/02	1200	K	JEB/ DRM	SQKICK	4/2/02	PAA	4/4/02	JEB	4/5/02	5/5/02

Figure 14: Macroinvertebrate Sample ID Log

Protocol I - Subsampling Procedures for Semi-Quantitative Samples

Biologist/Environmental Specialist with documented expertise in sorting.

All semi-quantitative samples are to be reduced to a 200+/- 20% (160-240) organism subsample using the following technique. This method comes directly from section 7.3 (pages 7-9) of the 1999 guidance, Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers (EPA 841-B-99-002).

1. Thoroughly rinse the sample in a 500-micron mesh sieve to remove preservative and fine sediment. Large organic material (whole leaves, twigs, etc.) not removed in the field should be rinsed, visually inspected and discarded. It may be necessary to soak the sample contents in water for about 15 minutes to hydrate the benthic organisms, which will prevent them from floating on the water surface during sorting. If the sample was stored in more than one container, the contents of all containers for a given sample should be combined at this time. Gently mix the sample by hand while rinsing to make it homogenous.

2. Transfer the cleaned sample to a gridded pick subsampler (or similar apparatus). The subsampler is a white plastic cutting tray that measures approximately 18" x 12½" x 2¼." The tray is divided into 28, 2"x2" grids and marked with indelible ink. Note: it is preferable that a sieve insert or raised grid divider be used to separate the grids. Remove the animals and debris using a combination of scoop and transfer pipette.

3. If the debris will not fit in one tray, use two or more trays. Thoroughly mix the debris and divide equally between the trays. Sort the same grids for both trays. For example, if grid # 5 is randomly selected, both # 5 grids are picked. This will count as one grid out of 28.

4. Add enough water (or ethanol) to evenly distribute the debris. Gently shake and swirl the tray until the organisms are evenly distributed within the tray. Remove the excess water with a suction device (i.e. turkey baster with a 500 micron or smaller screen over the aperture), to the point where the sample is settled onto the bottom of the tray. If a raised grid insert is not being used, care should be taken not to pull organisms towards the area of suction.

5. Randomly select four numbers corresponding to squares (grids) within the gridded subsampling pan. Remove all material (organisms and debris) from the four grids and place the material into a dish or jar with a small amount of water. Use a magnifying light to make sure all organisms and debris were removed from the grids. Any organism that is lying over a line separating two grids is considered to be on the grid containing its head. If it is not possible to determine the location of the head (i.e. oligochaetes), the organism is considered to be in the grid containing most of the body.

6. If there appears to be 160-240 organisms (cumulative of the four grids) then subsampling is completed. If there appears to be fewer than 160 organisms, continue selecting grids one at a time until between 160 and 240 organisms are selected. If more than 240 organisms are contained in the first four grids, transfer the contents of the four grids to a second gridded pan. Randomly select grids for this second subsampling as was done for the first, sorting grids four (and then one) at a time until the second subsample contains 160-240 organisms. If it is estimated that the first four grids of the second subsample contain more than 240 organisms, transfer the four grids to another pan and conduct a third subsample. Continue creating subsamples until there are 160-240 organisms.

7. Transfer the subsample, a small amount at a time to a petri dish for sorting (removing organisms). Complete all sorting under a dissecting scope, removing and preserving all organisms in 80% ethanol. If the number of organisms from the four-grid subsample does not equal the specified number of 160-240, randomly choose a fifth grid and pick out all organisms in that grid. If the addition of the fifth grid fulfills the quota, than the subsampling is complete. If not, choose additional grids (one at a time) until the quota is reached or surpassed. All the organisms from the final grid that is randomly selected are removed even if the quota is reached midway through the picking of the grid.

   If, after microscopic sorting, more than 240 organisms are found, transfer all organisms to a small gridded dish (36 grids). Subsample by groups of first four and then one random grid until the target of 160-240 organisms is achieved.

8. Place the sorted debris in a separate container and preserve in 80% ethanol. Include both external and internal tags (Figures 2 and 3). Add the words "sorted debris" to the tag information. Save the remaining unsorted sample debris residue in a separate container labeled "sample residue". This container should include the original sample label and internal tag.

9. Place the sorted 160-240 organism subsample into a glass vial and preserve in 80% ethanol. Place an internal tag written in pencil on waterproof paper citing the log number, station ID, date collected and taxonomist inside each vial (Figure 4). Position the label so it can be read through the vial.

   All chironomids and oligochaetes in the subsample are to be identified individually (do not subsample and extrapolate). Mount slides in a permanent mounting media (i.e. CMCP-10). Label slides with the station id, date collected, taxonomist initials and slide box number.

10. After sorting is completed, record the appropriate information (log number, station ID, sorters initials, date sorted and the number of organisms found) in the QC logbook (Figure 8, Section II-C).

11. All organisms and debris shall be retained as separately preserved samples for at least 5 years.

Protocol J - Taxonomy of Semi-Quantitative Samples

Biologist/Environmental Specialist with documented expertise in macroinvertebrate taxonomy

Semi-quantitative samples are to be sent to the central lab for analysis. This is to be coordinated through the Planning and Standards Section.

1. Identify all organisms to genus except Acari, Nematoda, Hydra, Brachiobdellida, immature Tubificidae, Lumbriculidae and Nematomorpha using the primary taxonomic keys listed in Appendix D. Do (Secondary keys will only be used to assist with difficult specimens.) Do not count animals that are missing heads, exuviae, empty shells, empty caddis cases, or terrestrial life stages.

2. Calculate all biometrics at the specified level only. The primary keys will be updated as new literature is available. It is important that all taxonomists use the same primary keys for consistency in identification and nomenclature.

3. Record taxonomic nomenclature and number of organisms on a bench sheet. Header information that must be provided includes the log number, station ID number, date collected, time collected, samplers, sample source location, ecoregion, sample type, taxonomist and analysis date. Taxonomic information should include order, family and genus. An example is provided in Figure 15 (format can vary as long as all the information is provided).

STATION ID: HOHUM012.3SE	**LOG NUMBER:** C0203007
SOURCE: Hohum Creek	**COL. BY:** ABC/DEF
LOCATION: 100 yds u/s Hwy Q	**DATE COL:** 03/07/02
SAMPLE TYPE: SQKICK	**TIME:** 1700
ECOREGION: 68b (drains 68c)	**TAXONOMIST:** ABC **DATE:** 03/08/02

Order	**Family**	**Genus**	**Count**
Ephemeroptera	Heptageniidae	Epeorus	20
Ephemeroptera	Heptageniidae	Stenacron	30
Ephemeroptera	Leptophlebiidae	Paraleptophlebia	2
Plecoptera	Leuctridae	Leuctra	9
Trichoptera	Hydropsychidae	Cheumatopsyche	32

Figure 15: Example of taxonomic bench sheet

4. After identifying all taxa in the subsample, return them to the vial and add fresh preservative (80% ethanol). Initial, date and add the log number to the internal tag (Figure 10). Store the sample for a minimum of five years.

5. Mount chironomids, oligochaetes and other small organisms on slides for identification. Use a permanent mounting media such as CMCP-10 which clears the mount so a separate clearing agent is not necessary. Use round coverslips (12 mm) for small specimens. Place one organism under each coverslip. A maximum of 10 coverslips can be placed on each slide. Square coverslips (22mm) can be used to mount larger specimens. Place one to three organisms under each coverslip with a maximum of 3 coverslips per slide.

   Mount chironomid larvae so that their bodies are viewed laterally and their heads are viewed ventrally. Apply enough pressure to the coverslip so that the mandibles are opened exposing the mentum. The S1 setae, premandibles and pectin epipharyngias should also be visible. Mount oligochaetes laterally with minimal pressure. The mounting media should extend past the edges of the coverslip to allow for shrinkage during drying. Allow the slides to air-dry at least 24 hours before attempting identification. (A slide dryer can be used to dry mounts faster if desired). Label slides with the log number, station ID, date, taxonomist initials and slide box slot number. Keep labeled slides in a slide box a minimum of five years after completion of the study.

   Note: Do not subsample chironomids and oligochaetes further. Identify each chironomid and oligochaete in the 160-240 organism subsample.

Protocol K - Data Reduction of Semi-Quantitative Samples

Biologist/Environmental Specialist with experience in macroinvertebrate taxonomy

A macroinvertebrate index, based on seven biometrics, has been developed by the Division for use in semi-quantitative macroinvertebrate surveys (Arnwine and Denton, 2001). This index is based on ecoregional reference data and calibrated by bioregion. The calibrated scoring criteria can be used in all streams that fit the sample criteria for that region (habitat sampled, sampling protocol, stream size) and have at least 80% of their upstream drainage in the same bioregion.

For streams that do not meet the profile (for instance non-riffle streams in bioregions that are calibrated to a SQKICK sample, streams that have more than 20% of their upstream drainage in other bioregions or streams whose drainage area is larger or small than that specified in biocriteria), calculate the same seven biometrics. However, the index tables cannot be used for assessment, since these samples are not comparable to streams in the ecoregion reference database. Compare the biometrics to an appropriate upstream or watershed reference.

1. Using the raw benthic data from the semi-quantitative subsample (kick or bank), calculate a numerical value for each of the seven biometrics. Calculate all biometrics using taxa identified to the genus level except for specified taxa (Acari, Branchiobdellida, Nematomorpha, Nematoda, Hydra, immature Tubificidae, Lumbriculidae) or those too young or too damaged to identify to this level. Species identification is not to be used.

   a. **TR** (Taxa Richness)

      Total the number of distinct genera found in the subsample. Taxa that could only be identified to family are included only if it is probable that they are distinct from other taxa identified to genus within the family. (Document on taxa list if an unidentified organism is determined to be a distinct taxon.)

   b. **EPT** (Ephemeroptera Plecoptera Trichoptera Richness)

      Total the number of genera within the orders Ephemeroptera, Plecoptera and Trichoptera. Taxa that could only be identified to family are included only if they are the only taxon found in that family or it is probable that they are distinct from other taxa identified to genus within the family. (Document on taxa list if an unidentified organism is determined to be a distinct taxon.)

   c. **% EPT-Cheum** (EPT Abundance excluding *Cheumatopsyche* spp.)

$$\% \text{ EPT} = \frac{\text{Total (Ephemeroptera + Plecoptera + Trichoptera)} - \text{Cheumatopsyche}}{\text{Total number of individuals in the subsample}} \times 100$$

d. **%OC** (Percent oligochaetes and chironomids)

$$\%OC = \frac{\text{Total number of Oligochaeta + Chironomidae}}{\text{Total number of individuals in the subsample}} \times 100$$

e. **NCBI** (North Carolina Biotic Index includes tolerance scores from other indices found in EPA RBP when no value is available for NC). Family value is to be assigned when there is no genus level tolerance value.

$$NCBI = \Sigma \frac{x_i t_i}{N \text{ (exclusive if no } t_i\text{)}}$$

where: x_i = number of individuals within a taxon
t_i = tolerance value of a taxon (Appendix C)
N = total number of individuals in the subsample that have been assigned a tolerance value (exclude animals for which no tolerance value is assigned see following note).

Note: For new taxa, tolerance values found in the latest version of the North Carolina Department of Environment and Natural Resources QSSOP should be assigned http://h2o.enr.state.nc.us/esb/BAUwww/benthossop.pdf. If only species level tolerance values are available, genus level is determined by averaging species. If a North Carolina tolerance score has not been assigned for a taxon, regional tolerance values in appendix B of EPA's Rapid Bioassessment protocols may be substituted. (Barbour et. al., 1999). Order of preference is Southeast, Midwest, Upper Midwest, Mid-Atlantic then Northwest. If there is no genus level tolerance value for new taxon in any of these documents, the family level tolerance value found in appendix C should be used.

f. **% Clingers** (Percent contribution of organisms (primary for genus) that build fixed retreats or have adaptations to attach to surfaces in flowing water)

A list of taxa designated as clingers is located in Appendix C. Merritt and Cummins, 2008 is used as authority for determination of primary clingers (if multiple habits are listed, only those where clinger is listed first are used.)

$$\%\ Clingers = \frac{\text{Total number of clinger individuals}}{\text{Total individuals in the sample}} \times 100$$

g **%TNUTOL (% TN Nutrient Tolerant Organisms)**

$$\% \text{ TNUTOL} = \frac{\text{Total number of Cheumatopsyche, Stenelmis, Polypedilum, Cricotopus, Cricotopus/Orthocladius, Lirceus, Caenis, Elimia, Nais, Dero, Undetermined (immature) Tubificidae}}{\text{Total individuals in the sample}} \times 100$$

After calculating values for the seven biometrics, equalize the data by assigning a score of 0, 2, 4 or 6 based on comparison to the ecoregion reference database for the bioregion and stream size (Appendix A). Total the seven scores to calculate the TMI (Tennessee Macroinvertebrate Index). **A score of 32 or higher is considered to pass biocriteria guidelines in all ecoregions except 73 where a score of 22 is passing (EPT Richness and % Clingers is not calculated in ecoregion 73).**

<u>Alternative Reference Stream Method</u>

Some sites may not meet the conditions necessary for comparison to the biocriteria tables in Appendix A. This will happen when:

a. The streams has less than 2 square miles drainage upstream of the sample site and headwater guidelines have not yet been developed for that bioregion. Drainage area size can be determined at http://water.usgs.gov/osw/streamstats/tennessee.html.

b. The stream is less than 80% within a bioregion upstream of the sample site.

c. The stream does not naturally have the habitat specified for comparison (for example low gradient non-riffle streams in bioregions where riffle criteria are specified. However, streams where the riffle is buried in sediment or otherwise compromised due to human disturbance should still be considered riffle streams).

In these cases, an alternative reference sample should be collected. The reference can be upstream, within the same watershed or within the same bioregion. Reference site selection should follow the same guidelines used for selection of ecoregion reference streams as specified in Protocol M. The alternative reference should be collected at the same time and using the same method (SQKICK or SQBANK) used at the test site.

Once the reference sample is collected, scoring ranges for each metric will be calculated based on quadrisection of the reference values for each metric. Reference data and scoring table, including ranges calculated for each metric, should be included with data report.

For metrics that were expected to decrease with increased pollution (TR, EPT, %EPT-Cheum, %Clingers):

Score 6: ≥ reference value –Reference value/4

Score 4: < lowest possible 6 to lowest possible 6 – reference/4

Score 2: < lowest possible 4 to lowest possible 4 – reference/4

Score 0: < lowest possible 2

For example:
If there were 30 distinct taxa found at the reference site, the scoring for taxa richness would be calculated by

Score 6: 30 – (30/4) or ≥ 22
Score 4: 22 – (30/4) or 14 to 21
Score 2: 14 - (30/4) or 6 to 14
Score 0: < 6

For metrics that were expected to increase with increased pollution (%OC, NCBI, %TNUTOL):

Score 6: < reference value + (highest possible value for metric – reference value)/4

Score 4: > highest possible 6 to highest possible 6 + (highest possible value for metric – reference value)/4

Score 2: > highest possible 4 to 0.1 + highest possible 4 + (highest possible value for metric – reference value)/4

Score 0: > highest possible 2

For example, if the %OC at the reference site was 20%,

Score 6: ≤ 20% + [(100% - 20%)/4] or ≤ 40.0%
Score 4: 40% + (100% - 20%)/4 or 40.1% to 60.0%
Score 2: 60% + (100% - 20%)/4 or 60.1% to 80.0%
Score 0: > 80.0%

Total the 7 scores to calculate the TMI. A score of 32 or higher is considered to pass biocriteria guidelines in all ecoregions except 73 where a score of 22 is passing (EPT Richness and % Clingers is not calculated in ecoregion 73).

Protocol L: Report Preparation

Biorecons

Biorecon results including biorecon field sheet, with final identifications and scores, habitat assessment field sheets, stream survey field sheets should be sent to the Planning and Standard section as soon after completion of assessment as possible. Data packets may be submitted hard copy or electronically with each survey in a separate file. Photos should be sent electronically at the end of the fiscal year, prior to assessments. File names should incorporate station ID and date.

Semi-quantitative Samples

a. Field office

   As soon after collection as possible, submit all samples and paperwork (sample request form/COC, habitat assessments, stream survey field sheet) to the state laboratory sample coordinator. Field office should maintain copy of all paperwork and voucher specimens for a minimum of five years.

b. Aquatic Biology Section – State Laboratory

   After sample completion and QC (by agreed due date) send copies of macroinvertebrate assessment field sheet, taxonomic lists, stream survey field sheet, habitat assessment field sheet, sample request form/COC, and any other paperwork such as printed pictures that were submitted with the sample to the Planning and Standards Section and appropriate field office. Electronic submittal is acceptable.

   Results should not be submitted until QC is complete. However, data should not be held past due date. Instead, QC should be completed prior to completion of group of 10. If the last sample QC'd fails, the next group of 10 should start after the failed sample. If the sample passes, additional QC does not need to be done until 10 samples are completed for that group.

   Taxonomic data should be entered in the SQSH database for metric calculation and electronic storage. The database should be sent to the Planning and Standard Section quarterly.

Photographs

Each field office should provide the Planning and Standards Section with a CD/DVD containing pictures of sample locations at the end of each fiscal year prior to assessments. File names should incorporate station ID and date.

Protocol M: Reference stream selection

The following guidelines are for selection of ecoregion reference screams. When selecting a project specific reference sites for atypical test streams the same guidelines should apply. Stream size, habitat, gradient and geology should be similar to the test site.

- Stream type, flow regime and substrate are typical of the Level IV ecoregion (or test site for atypical streams). Perennial streams will be targeted. Intermittent streams may be selected if this is typical of headwaters for the ecoregion.

- Streams are in a protected watershed or upstream land-use is primarily forested. In heavily urban or agricultural bioregions, stations will be selected where upstream watershed is least disturbed and is comparable to percent forested of other established reference streams.

- Riparian vegetation zone is well established with all size classes represented. Invasives constitute less than 10% of streamside vegetation.

- The upstream watershed does not contain a municipality, mining area or permitted discharger and is not heavily impacted by nonpoint source or other non-regulated source of pollution.

- Upstream drainage is at least 80% within a single Level IV ecoregion.

- The stream flows in its natural channel. There are no flow or water level modification structures such as dams, irrigation canals or field drains.

- No power lines or pipelines or any structure that is routinely cleared crosses upstream of the monitoring station.

- The upstream watershed contains few or no roads.

I.J - DATA AND RECORDS MANAGEMENT

Biorecon and semi-quantitative stations are established in the same Water Quality Database as chemical monitoring stations. The master database is kept on the H drive at the central office. The Planning and Standards Section is responsible for maintaining this database. The database is sent to the environmental field offices at the beginning of each quarter. The file name is Copy of wqlkcCO-EFO (current date). The Quality Team member or their designee in each EFO should check that all biological stations for their area have been entered with complete information and that duplicate sites do not exist under another station ID. Biorecon results are entered in the biorecon form of this database. Biological and chemical stations collected within 200 meters (yards) of each other are considered the same station and should be given the same station ID. (See station naming protocol for more information). After reviewing the stations table, an excel spreadsheet showing corrections should be sent to the Planning and Standards Section.

Taxa lists from semi-quantitative samples are entered by the Aquatic Biology Section, Lab Services, TDH in a 2000 Access file called SQDATA (date). Copies of taxa lists and habitat field sheets from any semi-quantitative samples identified by WPC Environmental Assistance Centers are to be sent to the Aquatic Biology Section for data entry. This database will be sent by the Aquatic Biology Section to the Planning and Standards Section at the completion of each project or quarterly as required. Calculated metrics for each sample will be entered by the PAS section in Water Quality Database.

Assessment information for each stream segment will be entered in the Assessment Database (ADB) by the Planning and Standards Section (PAS). PAS staff will meet with WPC managers and biologists in each EFO before assessments are finalized. This database is linked to a GIS map and is accessible on the web for public access: http://tnmap.tn.gov/wpc/

The original field sheets and taxa lists are kept in files at the sampling agency (Environmental Field Office or Aquatic Biology Section). Copies of all paperwork should accompany samples sent to the Aquatic Biology Section for analysis. The Aquatic Biology section will send copies of taxa lists, field and habitat forms, COC, and the macroinvertebrate assessment report to the PAS section and the appropriate field office. The Aquatic Biology Section will also send copies of all paperwork to the appropriate Environmental Field Office for projects where they collect the samples. For biorecons or other samples identified by the field offices, copies of the Stream Survey Field Sheet, Habitat Assessment Field Sheet and taxa lists along with Macroinvertebrate Assessment Report (Appendix B) are to be sent to the Planning and Standards Section. These copies will be kept in the watershed files for five years before being transferred to archive boxes. The Aquatic Biology Section will also keep copies of all paperwork and samples for five years after report date.

II. QUALITY ASSURANCE /QUALITY CONTROL

The U.S. EPA requires that a centrally planned, directed and coordinated quality assurance and quality control program be applied to efforts supported by them through grants, contracts or other formalized agreements. This time allocation is an essential component of biological sampling and analysis and will be included in annual work plans. This is not an optional or "as time allows" activity. The goal is to demonstrate the accuracy and precision of the biologists, as well as the reproducibility of the methodology, and to ensure unbiased treatment of all samples.

A. General QC Practices

1. Quality Team Leader (QC Coordinator) - A centralized biological QC coordinator will be designated with the responsibility to ensure that all QC protocols are met. This person will be an experienced water quality biologist in the Planning and Standards Section. Major responsibilities will include monitoring QC activities to determine conformance, distributing quality related information, training personnel on QC requirements and procedures, reviewing QA/QC plans for completeness, noting inconsistencies, and signing off on the QA plan and reports.

2. Quality Team Member (In-house QC officer) - One WPC biologist/environmental specialist in each EFO will be designated as the Quality Team Member (in-house QC officer.) This person will be responsible for performing and/or ensuring that quality control is maintained and for coordinating activities with the central Quality Team Leader (QC coordinator).

3. Training - Unless prohibited by budgetary travel restrictions, training will be conducted at least once a year through workshops, seminars and/or field demonstrations in an effort to maintain consistency, repeatability and precision between biologists/environmental specialists conducting macroinvertebrate surveys. This will also be an opportunity for personnel to discuss problems they have encountered with the methodologies and to suggest SOP revisions prior to the annual SOP review. Note: topics of discussion should be submitted to the central Quality Team Leader (QC coordinator) before the meeting so that a planned agenda can be followed, thus making the best use of limited time.

B. Field Quality Control – Habitat Assessment and Biological Sampling Methodology

1. Habitat Assessments - At minimally 10% of sites, two trained biologists/environmental specialists will complete habitat assessment field sheets independently. Scores are compared for each parameter with discrepancies arbitrated while in the field. A separate consensus field sheet should be submitted with both original data sheets. Circle on stream survey field sheet if duplicate and/or consensus completed.

2. Biorecon Collection A second biorecon will be collected at a minimum of 10% of the sites by a separate biologist/environmental specialist. This should be conducted at the same time,

or at least within two weeks of the original survey. If assessment does not agree (not-impaired, ambiguous, or impaired) biologists should investigate reason for disagreement. Results from the more representative sample should be used. If no reason could be determined for discrepancy, both biologists should collect another sample.

3. Semi-quantitative Sample - A second semi-quantitative sample will be collected at 10% of the sites within 2 weeks of the original sample. (If rain or other factor compromises reproducibility, the second sample should not be collected). Since this sampling method requires two people, it will not be possible in most offices for an independent team to conduct the sampling. Therefore, the same team can collect the samples with each investigator independently selecting the sample spot and performing the kick. At least once a year, a team from another EFO or the state lab should collect the QC sample.

   If assessment results do not agree (both scores above or below 32) the following action will take place until agreement is reached:

   a. The samplers will be contacted to determine if there was any discrepancy in habitat, location, environmental factors such as rain or collection methods. If so, the most representative sample will be used. This will not count as part of the required 10% duplicates.
   b. The lab will re-id both samples
   c. The lab will re-examine both subsamples for overlooked organisms and re-id
   d. The lab will re-subsample and id both samples
   e. Both taxa lists will be submitted to PAS. Taxa will be combined, statistically reduced to a 200 organisms subsample and re-scored. This will not count as a QC sample.

4. Chain of Custody Chain of custody is required by the TDEC Office of General Counsel for samples that have the potential of being used in court, reviewed by state boards, or involved in state hearings. Chain of custody must also accompany any contract samples (semi-quantitative samples being sent to the lab). Chain of custody is the far right column of the biological analysis form. (Appendix B) The entire form must be filled out completely.

   The chain of custody follows the sample through collection, transfer, storage, taxonomic identification, quality assurance and disposal. The biologist who collected the sample must sign (not print) their name in full (not initial) in the Collected By space with the date and time (24-hour clock). If the sample is given to anyone else before it is delivered to the lab, each person responsible for the sample must sign their full name on the Received By space with the date and time. The person in the laboratory who receives the sample will sign line four. The person who logs the sample in signs the last line.

C. QC Log

A list of all samples sorted and/or identified by each biologist/environmental specialist will be kept in a bound log (or electronically with electronic backup on a separate system) so that QC requirements and results can be documented (Figure 16). The QC log must contain the following information:

1. Sample log number
2. Station ID
3. Sample type
4. Initials of taxonomist and sorter
5. Number of organisms picked in subsample (semi-quantitative samples only)
6. Date completed
7. Initials of person performing QC
8. Number of organisms found in re-pick (semi-quantitative samples only)
9. Percent sorting efficiency (semi-quantitative samples only).
10. Date of QC identification
11. Initials of QC taxonomist
12. Results of taxonomic QC (satisfactory/unsatisfactory)

Log #	Station ID	Sample Type	Sort By	Sort Date	# org.	Sort QC	QC Date	QC # org.	Sort Eff.	S/U	ID By	ID Date	QC ID	QC Date	S/U
J0201001	BIFFL003.0DY	SQKICK													
J0201002	BIGGS000.7WY	SQKICK	AJF	3/11/02	190	PDS	3/20/02	10	95%	S	AJF	3/11/02	PDS	3/20/02	S
J0201003	BMHOL002.0OB	SQKICK													
J0201004	CANE001.8WY	SQKICK													
J0201005	CGROU001.2WY	SQKICK													
J0201006	CLEAR001.2HN	SQKICK													
J0201007	CLOVE001.4OB	SQKICK													
J0201008	CSPRI002.4DY	SQKICK													
J0202001	CYPRE00.6WY	SQKICK													
J0202002	CYPRE000.6OB	SQKICK													
J0202003	DAVID002.6OB	SQKICK													
J0202004	GRASS000.8OB	SQKICK													
J0203001	HFORK006.8OB	SQKICK													
J0203002	HOOSI000.5OB	SQKICK													
J0203003	HURRI002.6WY	SQKICK													
J0203004	HURRI003.9WY	SQKICK													
J0203005	HURRI1T1.1WY	SQKICK													
J0203006	MILL004.0OB	SQKICK													
J0203007	NFOBI005.9OB	SQKICK	AJF	3/15/02	220	PDS	3/20/02	14	94%	S	AJF	3/15/02	PDS	3/20/02	S
J0203008	NFOBI018.0WY	SQKICK													
J0203009	NFOBI026.5WY	BR													
J0203010	NFOBI040.6HN	BR	NA	NA	NA	NA	NA	NA	NA	NA	AJF	3/16/02	PDS	3/20/02	S
J0203011	OBION020.9DY	BR													
J0203012	OBION044.3DY	BR													

Figure 16: Example of macroinvertebrate QC log

D. Sorting Efficiency (Semi-Quantitative samples only)

1. Each biologist/ES responsible for sample sorting, regardless of previous experience, will have every sample QC'ed by a second biologist/ES who has already achieved 90% sorting efficiency (documented) until the original biologist/ES has passed 90% sorting efficiency on a sample. A record of this is kept in the QC log. Once a biologist/ES has passed their first QC, they are QC'ed on 10% of subsequent samples (randomly selected).

2. Each biologist/ES involved in sorting of semi-quantitative benthic macroinvertebrate samples will have 10% of their subsamples (debris) resorted by a second experienced biologist/ES. The sample to be QC'ed is randomly chosen by the person performing the QC after every group of 10 samples has been completed. (Or fewer if less than 10 are completed before due date). A sorting efficiency of 90% must be maintained. If fewer than 90% of the organisms are recovered, every sample prior to that one in the same group of 10 is resorted until a sample that has met the 90% requirement is found. The next group of 10 starts after the unsatisfactory sample.

   The sorting efficiency is calculated by:

   $$\text{Sorting efficiency} = \frac{\text{\# organisms found in initial pick}}{\text{Total \# organisms, both picks}} \times 100$$

   If fewer than 90% of organisms are found, the additional animals are added to the final ID of the first pick. If this puts the final subsample over 240 organisms transfer all organisms to a small gridded dish (36 grids). Subsample by groups of first four and then one random grid until the target of 160-240 organisms is achieved. If permanent mounts have already been made of the first pick, a 200 orgnism subsample should be calculated statistically:

   $$\text{Taxon a} = 200 * \frac{\text{\# taxon a found in both picks}}{\text{Total \# organisms both picks}}$$

   Round to nearest whole number.

3. Log results in the QC log.

4. All sorting QC must be completed before the data are released to ensure accuracy of results. However samples, should not be held up waiting for QC. Instead QC should be performed prior to completion of group of 10. If, for any reason, a report is released prior to QC completion, an addendum will be sent to all report recipients with any corrected information after QC is complete.

5. All subsample debris is preserved in 80% ethanol and kept in a labeled container for a minimum of 5 years. The original sample, from which the subsample was taken, is kept in a separate labeled container for the same period.

E. Taxonomic Verification

1. All biologists/ES are to be trained and show proficiency for genus level identification of each group of organisms. (Except Acari, Nematoda, Nematomorpha, Branchiobdellida, Enchytraeidae and Lumbriculidae). If the biologist/ES will only be performing family level biorecons, they need only demonstrate proficiency at the family level.

2. The same taxonomic keys for each group will be used by all taxonomists. Consistency in taxonomic keys is essential because couplets in different keys can result in identification discrepancies through differences in nomenclature or inconsistency of characters used for separation of taxa. This is particularly true where genera may exhibit great variability due to regionalization. Approved keys are listed in Appendix D. Keys will be updated continually as new literature is made available. However, biologists/ES should not utilize new keys for anything other than supplemental information until they are approved by the central QC coordinator and incorporated into the SOP to be used by all Division biologists/ES. New literature will be discussed at the annual training meetings. If new keys are approved for use as primary keys, taxa from reference stations will be reviewed to ensure that the most recent nomenclature is being used for reference information.

3. Each new biologist/ES, regardless of previous experience, will have every sample QC'ed by another biologist/ES (who has passed QC) until they have satisfactorily completed taxonomic QC on a sample. A record of this is kept in the QC log. Once a biologist/ES has satisfactorily completed their first QC, 10% of their identified samples will be randomly checked by another biologist/ES. The sample to be QC'ed is randomly chosen by the QC'er after every 10th sample is completed (or fewer depending on due date). The biologist/ES performing the QC will identify every organism in the sample without consulting the original taxonomist's list. Once the second identification is complete, the two biologists/ES will go over any discrepancies together.

4. To satisfy QC requirements for Semi-Quantitative samples, the two taxa lists must not show a significant difference as measured by a chi-square test utilizing a contingency table at alpha 0.05. If a sample shows a significant difference, every sample prior to that one in the same group of 10 will be checked until a sample that has met the QC requirement has been found. The next group of 10 will start after the unsatisfactory sample. For biorecons, assessment results must be the same (supporting, ambiguous or non-supporting) to pass QC. (Note for family level biorecons where complete vouchers are not collected on every sample, taxonomic QC will be on every 10th sample instead of random).

   Note: If both taxonomic QC is performed on the same sample where sorting efficiency is checked, only the animals found in the original pick will be used for QC.

5. Log results in the QC logbook.

6. Complete all taxonomic QC before releasing results ensure accuracy of results. Reports should never be held waiting for QC, if necessary perform QC prior to completion of group of 10. If, for any reason, results are released prior to QC completion, send an addendum to all report recipients with any adjusted information after QC is complete.

F. Voucher Collections

1. Family Level Biorecons – Representative specimens of families difficult to field identify, should always be collected for dissecting scope verification. Voucher collections including representatives of each taxon must be collected at a minimum of 10% of sites (randomly selected) for verification by dissecting scope. If mis-identifications occur, vouchers should be collected at all sites for those families missed until biologist has demonstrated proficiency in field identifications for that taxon.

2. Genus level – Vouchers collections including representatives of each taxon must be collected at every site for verification by dissecting scope.

3. All voucher samples must be maintained for five years.

G. Reference Collections

1. The designated QC officer (quality team member) for each EFO will maintain a permanent reference collection consisting of all taxa identified by that office. In addition, a master collection of all taxa identified in the state will be kept in the central laboratory. The organisms in the centralized master reference collection will be verified by outside experts recognized for expertise in a particular taxonomic group.

2. A list of verified organisms found in the state is provided in Appendix E. If new organisms, not on the verified state taxa list are identified by the EFO, the quality team member will send a representative of that taxon to the central laboratory. The laboratory will have the new taxon verified by an outside expert, will add the organism to the central reference collection and will notify all regional offices and PAS of its addition to the verified taxa list for Tennessee. Experts used for verification must meet the qualifications provided in Appendix E.

3. Each EFO and central laboratory reference collection will be catalogued with discrete collection numbers assigned to each taxon in each facility. Assign unique numbers that identify the reference location to specimens as they are added into the collection. For example, if sequential numbering is used, N0001 would be the first specimen in the Nashville EFO collection. Maintain an accession catalog of all reference material in a permanently bound log or electronic format with backup on a separate system. Each entry must contain the following information:

Accession number (This must be unique for each group of organisms in each collection)
Complete name (genus, authority = who id'd or verified it, date identified)
Higher taxa (family, order, class)
Locality data (Waterbody, location, county, ecoregion, station id)
Sample habitat
Name of collector/date of collection
Name of taxonomist
Name of verifier if appropriate
Number of specimens

4. Arrange specimens for ease of use, (according to accession number or in phylogenetic order). Retain wet specimens in 80% ethanol in small screw cap vials with rubber or Teflon lined caps or rubber stoppered vials. Retain large specimens in appropriate size specimen jars sealed with electrical tape to reduce evaporation. Inspect vials monthly for evaporation adding 80% ethanol as needed. Keep permanently mounted microscope slides in a slide storage box. Seal the edges of the coverslips to prevent shrinkage of media over time (clear nail polish works well).

5. Clearly label all reference vials and slides. Place the labels in the vial with wet specimens or attach to slides for mounted specimens. Label information at a minimum must include:

   Full name of the organism (Order, family, genus)
   Accession number (reference number)
   Station ID number
   Ecoregion
   Collection date
   Collector
   Taxonomist
   Verifier.

H. Data Reduction QC

1. Store raw data (non-manipulated) in one or more separate locations and in an electronic database with backup.

2. A second staff member checks all computer data entry correctness by direct comparison with the field or laboratory handwritten data sheets. The person performing the data entry QC initial and dates each page of the checked printout in red ink

3. A second staff member checks ten percent of all biometrics that were hand calculated. If an error is found, all of the calculations for that biometric in that sample set are checked. The person performing the QC initials and dates each checked metric in red ink.

4. Keep QC information in a file with the other information for that project a minimum of five years.

5. Complete all data reduction QC before results are released.

III. REFERENCES

Arnwine, D.H. and G.M. Denton. 2001. *Development of Regionally-based Interpretations of Tennessee's Existing Biological Integrity Criteria*. Tennessee Department of Environment and Conservation, Division of Water Pollution Control. Nashville. TN.

Arnwine, D.H. and G.M. Denton. 2001. *Habitat Quality of Least-Impacted Streams in Tennessee*. Tennessee Department of Environment and Conservation, Division of Water Pollution Control. Nashville. TN.

Barbour, M.T., Gerritsen, J., Snyder B.D. and J.B. Stribling. 1999. *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers*. EPA 841-B-99-002. U.S. Environmental Protection Agency, Office of Water, Washington D.C.

Brumley J.F., G.J. Pond and M.C Compton. 2003. *Determining Nutrient Impairment Using Biological and Other Non-Chemical Indicators in Kentucky Streams.* Kentucky Department for Environmental Protection. Division of Water, Frankfurt, Kentucky.

Griffith, G.E., J.M. Omernik and S. Azevedo. 1997. *Ecoregions of Tennessee*. EPA/600/R-97/022. NHREEL, Western Ecological Division, U.S. Environmental Protection Agency, Corvalis, Oregon.

Klemm, D.J., P.A. Lewis, F. Fulk, and J.M. Lazorchak. 1990. *Macroinvertebrate Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters*. EPA/600/4-90/030. U.S. Environmental Protection Agency, Office of Research and Development, Cincinnati, Ohio.

Lenat. D.R. 1993. *A Biotic Index for the Southeastern United States: Derivation and List of Tolerance Values, With Criteria for Assigning Water Quality Ratings.* Journal of the North American Benthological Society. 12(3):279-290.

Merritt, R.W., K.W., K.W. Cummins, and M.B. Berg (eds.). 2008. An Introduction to the Aquatic Insects of North America, 4th edition revised. Kendall/Hunt Publishing Co., Dubuque, IA. 1158 pp.

North Carolina Department of Environment and Natural Resources. 2007. *Standard Operating Procedures for Benthic Macroinvertebrates.* Biological Assessment Unit, Raleigh, NC.

Tennessee Department of Environment and Conservation. 2011. *Quality Assurance Project Plan for 106 Monitoring.* Division of Water Pollution Control, Nashville, TN.

Tennessee Department of Environment and Conservation. 2009. *Quality System Standard Operating Procedure for Chemical and Bacteriological Sampling of Surface Water*. Division of Water Pollution Control, Nashville, TN.

Tennessee Department of Environment and Conservation. 2006. *Quality System Standard Operating Procedure for Macroinvertebrate Stream Surveys*. Division of Water Pollution Control, Nashville, TN.

Tennessee Department of Environment and Conservation. 1996. *Tennessee Biological Standard Operating Procedure Manual, Vol. I: Freshwater Aquatic Macroinvertebrates*. Division of Water Pollution Control, Nashville, TN.

Tennessee Department of Health. 1993. *Macroinvertebrate Standard Operating Procedures*. Division of Laboratory Services, Nashville TN.

Tennessee Water Quality Control Board. 2008. *Rules of the Tennessee Department of Environment and Conservation Division of Water Pollution Control. Chapter-4-3. General Water Quality Criteria*. Tennessee Department of Environment and Conservation, Nashville, TN.

APPENDIX A

ECOREGION REFERENCE INFORMATION

BIOCRITERIA TABLES
ECOREGION REFERENCE STREAMS
HEADWATER ECOREGION REFERENCE STREAMS
REGIONAL EXPECTATIONS FOR INDIVIDUAL HABITAT PARAMETERS

Headwater data are preliminary. Caution should be used when using these tables to assess streams < 2 sq miles. Biocriteria tables are not provided for headwater streams (< 2 sq mile drainage) in 68b, 73a or 74a due to lack of sufficient reference data.

Bioregion: 65abei - 74b* Season: January – December Target TMI = 32 Scoring calibrated to 160-240 organism sample			Method = SQBANK Drainage > 2 sq mile (includes non-wadeable) Genus Level Identification	
Metric	6	4	2	0
Taxa Richness (TR)	> 36	25 – 36	12 – 24	< 12
EPT Richness (EPT)	> 9	7 – 9	3 – 6	< 3
% EPT-Cheum	> 35.3	23.6 – 35.3	11.8 – 23.5	< 11.8
% OC	< 46.3	46.3 – 64.2	64.3 – 82.1	> 82.1
NCBI	< 6.24	6.24 – 7.49	7.50 – 8.74	> 8.74
% Clingers	> 27.7	18.5 – 27.7	9.3 – 18.4	< 9.3
% TNutol	< 27.9	27.9 – 51.9	52.0 – 75.9	> 75.9

Bioregion: 65abei Season: January – December Target TMI = 32 Scoring calibrated to 160-240 organism sample			Headwater (draft use caution) Method = SQBANK Drainage ≤ 2 sq mile Genus Level Identification	
Metric	6	4	2	0
Taxa Richness (TR)	> 34	23 – 34	11 – 22	< 11
EPT Richness (EPT)	> 5	4 – 5	2 – 3	< 2
% EPT-Cheum	> 41.0	27.4 – 41.0	13.7 – 27.3	< 13.7
% OC	< 42.0	42.0 – 61.4	61.5 – 80.7	> 80.7
NCBI	< 5.20	5.20 – 6.79	6.80 – 8.40	> 8.40
% Clingers	> 17.1	11.5 – 17.1	5.8 – 11.4	<5.8
% TNutol	< 26.4	26.4 – 50.9	51.0 – 75.4	> 75.4

Bioregion: 74b Season: January – December Target TMI = 32 Scoring calibrated to 160-240 organism sample			Headwater (draft use caution) Method = SQBANK Drainage ≤ 2 sq mile Genus Level Identification	
Metric	6	4	2	0
Taxa Richness (TR)	> 29	20 – 29	10 – 19	< 10
EPT Richness (EPT)	> 2	2	1	0
% EPT-Cheum	> 6.8	4.6 – 6.8	2.3 – 4.5	< 2.3
% OC	< 67.9	67.9 – 78.5	78.6 – 89.3	> 89.3
NCBI	< 6.20	6.20 – 7.47	7.48 – 8.74	> 8.74
% Clingers	> 13.6	9.2 –13.6	4.6 – 9.1	< 4.6
% TNutol	< 28.0	28.0 – 52.0	52.1– 76.0	> 76.0

Bioregion 65j Season: January – December Target TMI = 32 Scoring calibrated to 160-240 organism sample			Method = SQKICK Drainage: All wadeable streams (caution < 2 square miles) Genus Level Identification	
Metric	6	4	2	0
Taxa Richness (TR)	> 31	21– 31	11– 20	< 11
EPT Richness (EPT)	> 11	8 – 11	4 – 7	< 4
% EPT-Cheum	> 44.5	29.7– 44.5	14.9 – 29.6	< 14.9
% OC	< 36.6	36.6 – 57.6	57.7 – 78.8	> 78.8
NCBI	< 4.79	4.79 – 6.52	6.53 – 8.26	> 8.26
% Clingers	> 49.7	33.2 – 49.7	16.6 – 33.1	< 16.6
% TNutol	< 27.9	27.9 –51.9	52.0 –75.9	> 75.9

Bioregion 66deik Season: January – December Target TMI = 32 Scoring calibrated to 160-240 organism sample			Method = SQKICK Drainage: All wadeable streams (caution < 2 square miles) Genus Level Identification	
Metric	6	4	2	0
Taxa Richness (TR)	> 32	22 – 32	11 – 21	< 11
EPT Richness (EPT)	> 15	10 – 15	5 – 9	< 5
% EPT-Cheum	> 56.9	38.0 – 56.9	19.0 – 37.9	< 19.0
% OC	< 30.4	30.4 – 53.5	53.6 – 76.7	> 76.7
NCBI	< 4.12	4.12 – 6.07	6.08 – 8.03	> 8.03
% Clingers	> 56.0	37.4 – 56.0	18.7 – 37.3	< 18.7
% TNutol	< 25.5	25.5 – 50.3	50.4 – 75.2	> 75.2

Bioregion: 66fgj Season: January – December Target TMI = 32 Scoring calibrated to 160-240 organism sample			Method = SQKICK Drainage: All wadeable streams (caution < 2 square miles) Genus Level Identification	
Metric	6	4	2	0
Taxa Richness (TR)	> 33	23 – 33	12 – 22	< 12
EPT Richness (EPT)	> 15	11–15	5 –10	< 5
% EPT-Cheum	> 50.5	33.7– 50.5	16.9 – 33.6	< 16.9
% OC	< 31.5	31.5 – 54.2	54.3 – 77.1	> 77.1
NCBI	< 4.32	4.32 – 6.21	6.22 – 8.10	> 8.10
% Clingers	> 58.8	39.3 – 58.8	19.6 – 39.2	< 19.6
% TNutol	< 26.2	26.2 – 50.8	50.9 – 75.4	> 75.4

Bioregion 67fghi Season: January – December Target TMI = 32 Scoring calibrated to 160-240 organism sample			Method = SQKICK Drainage > 2 sq mile Genus Level Identification	
Metric	6	4	2	0
Taxa Richness (TR)	> 28	19 – 28	10 – 18	< 10
EPT Richness (EPT)	> 9	7 – 9	4 – 6	< 4
% EPT-Cheum	> 44.7	29.9 – 44.7	14.9 – 29.8	< 14.9
% OC	< 27.3	27.3 – 51.4	51.5 – 75.7	> 75.7
NCBI	< 4.87	4.87 – 6.57	6.58 – 8.29	> 8.29
% Clingers	> 54.3	36.3 – 54.3	18.1 – 36.2	< 18.1
% TNutol	< 30.1	30.1 – 53.3	53.4 – 76.7	> 76.7

Bioregion: 67fghi Season: January – December Target TMI = 32 Scoring calibrated to 160-240 organism sample			Headwater (draft use caution) Method = SQKICK Drainage ≤ 2 sq mile Genus Level Identification	
Metric	6	4	2	0
Taxa Richness (TR)	> 30	20 – 30	10 – 19	< 10
EPT Richness (EPT)	> 9	7– 9	4 – 6	< 4
% EPT-Cheum	> 45.4	30.3 – 45.4	15.2 – 30.2	< 15.2
% OC	< 28.4	28.4 – 52.2	52.3 – 76.1	> 76.1
NCBI	< 4.68	4.68 – 6.45	6.46 – 8.23	> 8.23
% Clingers	> 49.5	33.1– 49.5	16.6 – 33.0	< 16.6
% TNutol	< 26.4	26.4 – 50.9	51.0 –75.4	> 75.4

Bioregion 68a Season: January – June Target TMI = 32 Scoring calibrated to 160-240 organism sample			Method = SQKICK Drainage: All wadeable streams (caution < 2 square miles) Genus Level Identification	
Metric	6	4	2	0
Taxa Richness (TR)	> 31	21 – 31	11 – 21	< 11
EPT Richness (EPT)	> 11	8 – 11	4 – 7	< 4
% EPT-Cheum	> 48.3	32.3 – 48.3	16.2– 32.2	< 16.2
% OC	< 31.4	31.4 – 54.2	54.3 – 77.1	> 77.1
NCBI	< 4.72	4.72 – 6.47	6.48 – 8.24	> 8.24
% Clingers	> 45.8	30.6 – 45.8	15.3 – 30.5	< 15.3
% TNutol	< 26.8	26.8 – 51.1	51.2 – 75.6	> 75.6

Bioregion 68a Season: July - December Target TMI = 32 Scoring calibrated to 160-240 organism sample			Method = SQKICK Drainage: All wadeable streams (caution < 2 square miles) Genus Level Identification	
Metric	6	4	2	0
Taxa Richness (TR)	> 34	24 – 34	12 – 23	< 12
EPT Richness (EPT)	> 13	9 – 13	4 – 8	< 4
% EPT-Cheum	> 46.8	31.3 – 46.8	15.6 – 31.2	< 15.6
% OC	< 31.9	31.9 – 54.6	54.7 – 77.3	> 77.3
NCBI	< 4.85	4.85 – 6.56	6.57 – 8.28	> 8.28
% Clingers	> 57.9	38.6 – 57.9	19.3 – 38.5	< 19.3
% TNutol	< 28.3	28.3 – 52.2	52.3 – 76.1	> 76.1

Bioregion 68b Season: January - December Target TMI = 32 Scoring calibrated to 160-240 organism sample			Method = SQKICK Drainage: > 2 sq miles Genus Level Identification	
Metric	6	4	2	0
Taxa Richness (TR)	> 30	21 – 30	10 – 20	< 10
EPT Richness (EPT)	> 9	7 – 9	4 – 6	< 4
% EPT-Cheum	> 47.1	31.5 – 47.1	15.7 – 31.4	< 15.7
% OC	< 35.5	35.5 –56.9	57.0 – 78.5	> 78.5
NCBI	< 5.29	5.29 – 6.85	6.86 – 8.42	> 8.42
% Clingers	> 34.9	23.3 – 34.9	11.6 – 24.9	< 11.6
% TNutol	< 36.1	36.1 – 57.3	57.4 – 78.6	> 78.6

Subregion 68cd Season: January - June Target TMI = 32 Scoring calibrated to 160-240 organism sample			Method = SQKICK Drainage: All wadeable streams (caution < 2 square miles) Genus Level Identification	
Metric	6	4	2	0
Taxa Richness (TR)	> 26	18 – 26	9 – 17	< 9
EPT Richness (EPT)	> 11	8 – 11	4 –7	< 4
% EPT-Cheum	> 57.4	38.3 –57.4	19.2 –38.2	< 19.2
% OC	< 31.3	31.3 – 54.2	54.3 – 77.1	> 77.1
NCBI	< 4.64	4.64 – 6.42	6.43 – 8.21	> 8.21
% Clingers	> 42.4	28.3 – 42.4	14.2 – 28.2	< 14.2
% TNutol	< 27.0	27.0 –51.3	51.4 – 75.6	> 75.6

Subregion 68cd Season: July - December Target TMI = 32 Scoring calibrated to 160-240 organism sample			Method = SQKICK Drainage: All wadeable streams (caution < 2 square miles) Genus Level Identification	
Metric	6	4	2	0
Taxa Richness (TR)	> 26	18 – 26	9 – 17	< 9
EPT Richness (EPT)	> 11	8 – 11	4–7	< 4
% EPT-Cheum	> 40.5	27.1 – 40.5	13.5 – 27.0	< 13.5
% OC	< 30.9	30.9 – 53.9	54.0 – 76.9	> 76.9
NCBI	< 5.27	5.27 – 6.84	6.85 – 8.42	> 8.42
% Clingers	> 45.2	30.2 – 45.2	15.1 – 30.1	< 15.1
% TNutol	<31.4	31.4 – 54.2	54.3 – 77.2	> 77.2

Bioregion 69de Season: January - June Target TMI = 32 Scoring calibrated to 160-240 organism sample			Method = SQKICK Drainage: All wadeable streams (caution < 2 square miles) Genus Level Identification	
Metric	6	4	2	0
Taxa Richness (TR)	> 31	22 – 31	11 – 21	< 11
EPT Richness (EPT)	> 14	10 – 14	5 – 9	< 5
% EPT-Cheum	> 60.1	40.2 – 60.1	20.1 – 40.1	< 20.1
% OC	< 33.4	33.4 – 55.5	55.6 – 77.7	> 77.7
NCBI	< 3.93	3.93 – 5.95	5.96 – 7.98	> 7.98
% Clingers	> 54.1	36.1 – 54.1	18.1 – 36.0	< 18.1
% TNutol	< 25.7	25.7 – 50.4	50.5 – 75.2	> 75.2

Bioregion: 69de Season: July-December Target TMI = 32 Scoring calibrated to 160-240 organism sample			Method = SQKICK Drainage: All wadeable streams (caution < 2 square miles) Genus Level Identification	
Metric	6	4	2	0
Taxa Richness (TR)	> 34	24 – 34	12 – 23	< 12
EPT Richness (EPT)	> 12	9 – 12	4 – 8	< 4
% EPT-Cheum	> 55.5	37.1 – 55.5	18.5 – 37.1	< 18.5
% OC	< 31.9	31.9 – 54.6	54.7 – 77.3	> 77.3
NCBI	< 4.71	4.71 – 6.47	6.48 – 8.24	> 8.24
% Clingers	> 53.0	35.4 – 53.0	17.7 – 35.3	< 17.7
% TNutol	< 26.7	26.7 – 51.0	50.1 – 75.4	> 75.4

Bioregion 71e Season: January - December Target TMI = 32 Scoring calibrated to 160-240 organism sample			Method = SQKICK Drainage: All wadeable streams (caution < 2 square miles) Genus Level Identification	
Metric	6	4	2	0
Taxa Richness (TR)	> 26	18 – 26	9 – 17	< 9
EPT Richness (EPT)	> 9	7– 9	3 – 6	< 3
% EPT-Cheum	> 38.9	26.0 – 38.9	13.0 – 25.9	< 13.0
% OC	< 28.3	28.3– 52.1	52.2 – 76.0	> 76.0
NCBI	< 5.25	5.25 – 6.82	6.83 – 8.40	> 8.40
% Clingers	> 57.1	38.1 – 57.1	19.1 – 38.0	< 19.1
% TNutol	< 34.0	34.0 – 55.9	56.0 – 78.0	> 78.0

Bioregion 71fgh Season: January - December Target TMI = 32 Scoring calibrated to 160-240 organism sample			Method = SQKICK Drainage > 2 sq mile Genus Level Identification	
Metric	6	4	2	0
Taxa Richness (TR)	> 27	19 – 27	10 – 18	< 10
EPT Richness (EPT)	> 9	7 – 9	4 – 6	< 4
% EPT-Cheum	> 49.3	33.0 – 49.3	16.5 – 32.9	< 16.5
% OC	< 27.8	27.8 – 51.8	51.9 – 75.9	> 75.9
NCBI	< 4.88	4.88 – 6.58	6.59 – 8.29	> 8.29
% Clingers	> 55.3	37.0 – 55.3	18.5 – 36.9	< 18.5
% TNutol	< 29.3	29.3 – 52.8	52.9 – 76.4	> 76.4

Bioregion: 71fgh Season: January - December Target TMI = 32 Scoring calibrated to 160-240 organism sample			Method = SQKICK Drainage ≤ 2 sq mile Genus Level Identification	
Metric	6	4	2	0
Taxa Richness (TR)	> 28	19 – 28	9 – 18	< 9
EPT Richness (EPT)	> 11	8 –11	4 – 7	< 4
% EPT-Cheum	> 53.3	35.6 – 53.3	17.8 – 35.5	< 17.8
% OC	< 44.3	44.3 – 62.8	62.9 – 81.4	> 81.4
NCBI	< 5.47	5.47 – 6.98	6.99 – 8.49	> 8.49
% Clingers	> 57.4	38.3 – 57.4	19.2 – 38.2	< 19.2
% TNutol	< 45.6	45.6 – 63.8	63.9 – 81.9	> 81.9

Bioregion 71i Season: January - December Target TMI = 32 Scoring calibrated to 160-240 organism sample			Method = SQKICK Drainage > 2 sq miles Genus Level Identification	
Metric	6	4	2	0
Taxa Richness (TR)	> 23	16 – 23	8 – 15	< 8
EPT Richness (EPT)	> 7	5 – 7	2 – 4	< 2
% EPT-Cheum	> 36.3	24.3 – 36.3	12.1 – 24.2	< 12.1
% OC	< 34.2	34.2 – 56.1	56.2 – 78.1	> 78.1
NCBI	< 5.60	5.60 – 7.06	7.07 – 8.53	> 8.53
% Clingers	> 48.3	32.3 – 48.3	16.2 – 32.2	< 16.2
% TNutol	< 37.2	37.2 – 58.0	58.1 – 79.0	> 79.0

Bioregion 71i Season: January - December Target TMI = 32 Scoring calibrated to 160-240 organism sample			Method = SQKICK Drainage ≤ 2 sq mile Genus Level Identification	
Metric	6	4	2	0
Taxa Richness (TR)	> 15	11–15	5 – 10	< 5
EPT Richness (EPT)	> 6	4 – 6	2 – 3	< 2
% EPT-Cheum	> 43.4	29.0 – 43.4	14.5 – 43.4	< 14.5
% OC	< 31.9	31.9 – 54.5	54.6 – 77.3	> 77.3
NCBI	< 5.72	5.72 – 7.14	7.15 – 8.57	> 8.57
% Clingers	> 41.1	27.5 – 41.1	13.7 – 27.4	< 13.7
% TNutol	< 46.5	46.5 – 64.2	64.3 – 82.1	> 82.1

Bioregion 71i Season: January - December Target TMI = 32 Scoring calibrated to 160-240 organism sample			Method = SQBANK Drainage > 2 sq miles Genus Level Identification	
Metric	6	4	2	0
Taxa Richness (TR)	> 32	22 – 32	11 – 21	< 11
EPT Richness (EPT)	> 5	4 – 5	2 – 3	< 2
% EPT-Cheum	> 30.7	20.5 – 30.7	10.3 – 20.4	< 10.3
% OC	< 30.0	30.0 – 53.3	53.4 – 76.6	> 76.6
NCBI	< 6.89	6.89 – 7.92	7.93 – 8.96	> 8.96
% Clingers	> 21.5	14.4 – 21.5	7.2 – 14.3	< 7.2
% TNutol	< 34.5	34.5 – 56.3	56.4 – 78.1	> 78.1

Bioregion 73ab Season: January – December Target TMI = 22 Scoring calibrated to 160-240 organism sample			Method = SQBANK Drainage: All wadeable streams (caution < 2 square miles) Genus Level Identification	
Metric	6	4	2	0
Taxa Richness (TR)	> 25	17 – 25	8 – 16	< 8
EPT Richness (EPT)*	NA	NA	NA	NA
% EPT-Cheum	> 23.5	15.7 – 23.5	7.9 – 15.6	< 7.9
% OC	< 28.4	28.4 – 52.2	52.3 – 76.1	> 76.1
NCBI	< 7.73	7.73 – 8.47	8.48 – 9.23	> 9.23
% Clingers*	NA	NA	NA	NA
% TNutol	< 31.0	31.0 – 54.0	54.1 – 77.0	> 77.0

- EPT Richness and %Clingers are not calculated for TMI in Bioregion 73ab

Bioregion 74a Season: January – June Target TMI = 32 Scoring calibrated to 160-240 organism sample			Method = SQKICK Drainage > 2 sq miles Genus Level Identification	
Metric	6	4	2	0
Taxa Richness (TR)	> 19	13 - 19	7 – 12	< 7
EPT Richness (EPT)	> 5	4 – 5	2 – 3	< 2
% EPT-Cheum	> 27.2	18.2 – 27.2	9.1 – 18.1	< 9.1
% OC	< 59.7	59.7 – 73.1	73.2 – 86.5	> 86.5
NCBI	< 6.12	6.12 – 7.41	7.42 – 8.70	> 8.70
% Clingers	> 20.7	13.9 – 20.7	7.0 – 13.8	< 7.0
% TNutol	< 48.0	48.0 – 65.3	65.4– 82.6	> 82.6

Bioregion 74a Season: July - December Target TMI = 32 Scoring calibrated to 160-240 organism sample			Method = SQKICK Drainage > 2 sq miles Genus Level Identification	
Metric	6	4	2	0
Taxa Richness (TR)	> 22	16 – 22	8 – 15	< 8
EPT Richness (EPT)	> 5	4 – 5	2 – 3	< 2
% EPT-Cheum	> 43.4	29.0 –43.4	14.5 –28.4	< 14.5
% OC	< 30.0	30.0 – 53.3	53.4 – 76.6	> 76.6
NCBI	< 5.46	5.46 – 6.97	6.97 – 8.48	> 8.48
% Clingers	> 52.0	34.8 – 52.0	17.4 – 34.7	< 17.4
% TNutol	<45.2	45.3 – 63.4	63.5 –81.7	> 81.7

NOTE: ecoregion 74b is in bioregion 65abei-74b (first table) for streams greater than 2 square mile drainage and bioregion 74b for headwater streams (second table).

ECOREGION REFERENCE STREAMS

SITE #	STATUS	STREAM	USGS HUC	LOCATION	COUNTY	LATITUDE	LONGITUDE
ECO65B04	Active	Cypress Creek	08010207 Upper Hatchie	RM 5.5 U/S Buster King Rd	Hardeman	35.0675	-88.86
ECO65E04	Active	Blunt Creek	06040005 TN Western Valley	RM 0.1 U/S McHee Levee Rd	Carroll	35.95916	-88.26805
ECO65E06	Active	Griffin Creek	08010204 S Fk Forked Deer	RM 5 U/S Stanford Lane Ford	Carroll	35.81861	-88.54055
ECO65E08	Active	Harris Creek	08010201 N Fk Forked Deer	RM 2.2 Potts Chapel Rd	Madison	35.62638	-88.69972
ECO65E10	Active	Marshall Creek	08010208 Lower Hatchie	RM 2.2 Van Buren Rd	Hardeman	35.1619	-89.0694
ECO65E11	Probation	West Fork Spring Creek	08010208 Lower Hatchie	RM 1.7 U/S Van Buren Rd	Hardeman	35.10194	-89.08194
ECO65J04	Active	Pompeys Branch	06030005 TN Pickwick Lake	U/S Pompeys Branch Rd	Hardin	35.05388	-88.16805
ECO65J05	Probation	Dry Creek	06030005 TN Pickwick Lake	RM 3.2 Dry Creek Rd	Hardin	35.035	-88.15222
ECO65J06	Active	Right Fork Whites Creek	06040001 TN Western Valley	RM 3.4 U/S Morris Lane	Hardin	35.05305	-88.04777
ECO65J11	Active	Unnamed Trib. Rt Fork Whites Cr	06040001 TN Western Valley	RM 0.1 U/S Morris Lane	Hardin	35.05225	-88.04825
ECO66D03	Active	Laurel Fork	06010103 Watauga	RM 6.7 U/S Big Branch Off Dennis Cove Rd	Carter	36.2563	-82.10981
ECO66D05	Active	Doe River	06010103 Watauga	RM 26 U/S Picnic Area Roan Mtn State Park	Carter	36.15888	-82.10583
ECO66E04	Active	Gentry Creek	06010102 South Fork Holston	RM 2.1 Gentry Creek Rds end.	Johnson	36.5441	-81.7237
ECO66E09	Active	Clark Creek	06010108 Nolichucky	RM 1.8 National Forest property off Hwy 107 Clarks Creek Rd	Unicoi	36.14818	-82.52835
ECO66E11	Active	Lower Higgins Creek	06010108 Nolichucky	RM 1.7 Lower Higgins Cr Rd 1 mi NW Ernestville	Unicoi	36.08722	-82.52027
ECO66E17	Active	Double Branch	06010201 Fort Loudoun Lake	RM 0.1 U/S Millers Cove Rd	Blount	35.74378	-83.76631
ECO66F06	Active	Abrams Creek	06010204 Little Tennessee	RM 18.3 West end of Cades Cove, 0.6 mi U/S Mill Creek	Blount	35.59305	-83.84694

ECOREGION REFERENCE STREAMS

SITE #	STATUS	STREAM	USGS HUC	LOCATION	COUNTY	LATITUDE	LONGITUDE
ECO66F07	Active	Beaverdam Creek	06010102 S Fork Holston	RM 5, 1 mi SW Backbone Rock Park	Johnson	36.58638	-81.8275
ECO66F08	Active	Stony Creek	06010103 Watauga	RM 12.5 U/S SR 91	Carter	36.46811	-81.99569
ECO66G04	Active	Middle Prong Little Pigeon R	06010107 Lower French Broad	RM 0.5 U/S restricted rd 0.2 mi east Greenbriar Cove	Sevier	35.70666	-83.37888
ECO66G05	Active	Little River	06010201 Ft Loudoun/Little R	RM 50.7 U/S last house Little River Trail above Elkmont	Sevier	35.65333	-83.57727
ECO66G07	Active	Citico Creek	06010204 Little Tennessee	RM 5.2, one mile U/S Jakes Creek	Monroe	35.50555	-84.10694
ECO66G09	Active	North River	06010204 Little Tennessee	RM 3, 500 meters U/S campground on North River Rd	Monroe	35.32777	-84.14583
ECO66G12	Active	Sheeds Creek	03150101 Conasauga	RM 1.8, 0.25 mi U/S Shheds Creek Rd	Polk	35.00305	-84.61222
ECO67F06	Active	Clear Creek	06010207 Lower Clinch	RM 1, U/S Norris Municipal Park Road	Anderson	36.21361	-84.05972
ECO67F13	Active	White Creek	06010205 Upper Clinch	RM 2, D/S old USGS gauging station next to White Creek Rd	Union	36.34361	-83.89166
ECO67F14	Active	Powell River	06010206 Powell	RM 106.5 McDowell Shoal D/S Fourmile Creek	Hancock	36.57764	-83.3659
ECO67F16	Active	Hardy Creek	06010206 Powell	RM 0.5, U/S SR 660 Powell Valley Rd	Lee County, VA	36.6499	-83.2496
ECO67F17	Active	Big War Creek	06010205 Upper Clinch	RM 0.6 Pawpaw Rd	Hancock	36.42626	-83.34663
ECO67F23	Active	Martin Creek	06010206 Powell	RM 0.5 Powell Valley Rd just U/S Hopkins Rd	Hancock	36.59111	-83.335
ECO67F27	Candidate 5-5-09	Indian Creek	6010205 Clinch-Upper	Off Indian Creek Rd	Grainger	36.39519	-83.40339
ECO67G05	Active	Bent Creek	06010108 Nolichucky	RM 1.9 East of Hwy 340	Hamblen	36.18793	-83.16414
ECO67G08	Probation	Brymer Creek	06020002 Hiwassee	RM 1.3, U/S Spring Br off Roark Lane/Brymer Creek Rd	Bradley	35.12666	-84.96388

ECOREGION REFERENCE STREAMS

SITE #	STATUS	STREAM	USGS HUC	LOCATION	COUNTY	LATITUDE	LONGITUDE
ECO67G09	Active	Harris Creek	06020002 Hiwassee	RM 4.8, U/S Bancroft Rd	Bradley	35.175	-84.97916
ECO67G10	Active	Flat Creek	06010107 Lower French Broad	RM 12 D/S Muddy Hollow Rd	Sevier	35.9157	-83.4515
ECO67H06	Active	Laurel Creek	06010204 Little Tennessee	RM 0.8, D/S Lurel Creek Rd	Monroe	35.44829	-84.28833
ECO6701	Active	Big Creek	06010104 Holston	RM 9.8, D/S Fisher Creek West of Surgoinsville on Stanley Valley Rd	Hawkins	36.4778	-82.9387
ECO6702	Active	Fisher Creek	06010104 Holston	RM 0.6, U/S Bray Road	Hawkins	36.49	-82.94027
ECO6707	Probation	Possum Creek	06010102 South Fork Holston	RM 1.5, Weaver Pike Bridge, Bluff City	Sullivan	36.47964	-82.19932
ECO68A01	Probation	Rock Creek	05130104 S Fork Cumberland	RM 24.8, Pickett State Park	Pickett	36.57833	-84.79472
ECO68A03	Active	Laurel Fork of Station Camp Cr	05130104 S Fork Cumberland	RM 4, Big South Fork NRA	Fentress/ Scott	36.51611	-84.69805
ECO68A08	Active	Clear Creek	06010208 Emory	RM 4, Genesis Rd (HWY 298)	Morgan	36.11916	-84.7425
ECO68A20	Active	Mullens Creek	06020001 Tennessee	RM 5, U/S Jeep Trail	Marion	35.12472	-85.44388
ECO68A26	Active	Daddy’s Creek	06010208 Emory	RM 2.3, U/S Hebbertsburg Rd, Catoosa	Cumberland	36.05861	-84.79138
ECO68A27	Probation	Island Creek	06010208 Emory	RM 2.3, U/S Noah Hambrey Rd, Catoosa	Morgan	36.05138	-84.66805
ECO68A28	Active	Rock Creek	06010208 Emory	RM 1.8, Off Hwy 62 approx 1 mi NE Lancing	Morgan	36.13277	-84.64166
ECO68B01	Active	Crystal Creek	06020004 Sequatchie	RM 1.2, Approx 0.25 mi D/S Lower East Valley Rd	Bledsoe	35.54083	-85.21694
ECO68B02	Active	McWilliams Creek	06020004 Sequatchie	RM 1.9, D/S Smith Rd	Sequatchie	35.4175	-85.32083
ECO68B09	Active	Mill Branch	06020004 Sequatchie	RM 0.4, U/S Upper East Valley Rd	Bledsoe	35.67444	-85.08888

ECOREGION REFERENCE STREAMS

SITE #	STATUS	STREAM	USGS HUC	LOCATION	COUNTY	LATITUDE	LONGITUDE
ECO68C13	Active	Mud Creek	06030003 Upper Elk	RM 5.6, U/S E Roarks Cove Rd	Franklin	35.23055	-85.91722
ECO68C15	Active	Crow Creek	06030001 Guntersville Lake	RM 34.7, Off Ford Spring Rd below UT in Tom Pack Hollow	Franklin	35.1138	-85.9128
ECO68C20	Active	Crow Creek	06030001 Guntersville Lake	RM 35, Off Ford Spring Rd upstream UT in Tom Pack Hollow	Franklin	35.1155	-85.9110
ECO68C21	Active	Gilbreath Creek	06020001 Lower Tennessee	RM 0.1, Cove Loop Lower Rd Crossing	Rhea	35.47931	-85.07603
ECO69D03	Active	Flat Fork	06010208 Emory	RM 5, U/S Flat Fork Rd, U/S Rock Fork Branch	Morgan	36.1235	-84.5122
ECO69D05	Active	New River	05140104 S Fork Cumberland	RM 55.4, approx 0.5 mi U/S HWY 116, 0.3 mi U/S Morgan/Anderson Co. line	Morgan	36.12444	-84.43130
ECO69D06	Probation	Round Rock Creek	05130104 S Fork Cumberland	RM 1, U/S ford off Norma Rd	Campbell	36.24722	-84.28444
ECO69E01	Active	No Business Branch	05130101 Upper Cumberland	RM 0.2, U/S Hwy 25	Campbell	36.55277	-84.06861
ECO69E04	Active	Stinking Creek	05130101 Upper Cumberland	RM 15.1, Approx 0.5 mi south of Stinking Creek Rd near power line	Campbell	36.4258	-84.2618
ECO71E09	Active	Buzzard Creek	05130206 Red	RM 1.3, Buzzard Creek Rd	Robertson	36.60583	-86.98361
ECO71E14	Active	Passenger Creek	05130206 Red	RM 1.6, HWY 76	Montgomery	36.53444	-87.19583
ECO71E17	Candidate	Brush Creek	05130206 Red	Stroudville Rd	Robertson	36.481389	-87.089722
ECO71E18	Active	Santee Creek	05130206 Red	Sprouse Rd	Robertson	36.49778	-86.778333
ECO71E19	Active	Calebs Creek	05130206 Red	U/S Maxie/Carr Rd	Robertson	36.49237	-87.0066
ECO71F12	Probation	South Harpeth Creek	05130204 Harpeth	RM 16.9, South Harpeth and Pewitt Rd, U/S Kelley Creek	Williamson	35.925	-87.0929
ECO71F16	Active	Wolf Creek	06040003 Lower Duck	RM 0.8, U/S Wolf Creek Rd	Hickman	35.81805	-87.68527
ECO71F19	Active	Brush Creek	06040004 Buffalo	RM 2.1, Paul Reed Rd, U/S Little Brush Creek	Lewis/Lawrence	35.41972	-87.53416

ECOREGION REFERENCE STREAMS

SITE #	STATUS	STREAM	USGS HUC	LOCATION	COUNTY	LATITUDE	LONGITUDE
ECO71F27	Active	Swanegan Branch	06030005 Pickwick Lake	RM 0.5, Off Thomas Woodard Rd	Wayne	35.06916	-87.6375
ECO71F28	Active	Little Swan Creek	06040003 Lower Duck	RM 5.6, Meriwether Lewis National Monument	Lewis	35.52888	-87.45361
ECO71F29	Active	Hurricane Creek	06040003 Lower Duck	RM 6.6, Hwy 13	Humphreys	35.980556	-87.761389
ECO71G03	Active	Flat Creek	05130106 Upper Cumberland	RM 1.8, HWY 136	Putnam	36.35944	-85.43138
ECO71G04	Active	Spring Creek	05130106 Upper Cumberland	RM 16.2, Boatman Rd	Overton	36.27277	-85.42333
ECO71G10	Active	Hurricane Creek	06030003 Upper Elk	RM 9.4, Hurricane Creek Rd	Moore	35.32083	-86.29944
ECO71G14	Probation	Blackburn Fork	05130106 Upper Cumberland	Cummins Mill Rd	Jackson	36.2506	-85.5647
ECO71H03	Active	Flynn Creek	05130106 Upper Cumberland	RM 10.2, Flynn Creek Rd, 3 mi NE Nameless TN	Jackson	36.2792	-85.66444
ECO71H06	Active	Clear Fork	05130108 Caney Fork	RM 6.8, Off Big Hill Rd	Dekalb/Cannon	35.92416	-85.99083
ECO71H09	Active	Carson Fork	05130203 Stones	RM 4.2, Burt-Burgen Rd, 2 mi NE Bradyville	Cannon	35.76495	-86.13263
ECO71I10	Active	Flat Creek	06040002 Upper Duck	RM 6.4, U/S Hazelwood Rd	Marshall	35.68583	-86.80166
ECO71I12	Active	Cedar Creek	05130201 Cumberland	RM 4.6, Centerville Rd	Wilson	36.28425	-86.20339
ECO71I14	Active	Little Flat Creek	06040002 Upper Duck	RM 3.6, U/S Will Brown Rd	Maury	35.69903	-86.83872
ECO71I15	Active	Harpeth River	05130204 Harpeth	RM 105.7, D/S McDaniel Rd	Williamson	35.8325	-86.70019
ECO71I16	Active	West Fork Stones River	05130203 Stones	RM 30.4, Walnut Grove Rd	Rutherford	35.7225	-86.4451
ECO71I17	Candidate 4-23-09	Spring Creek	05130201 Cumberland Old Hickory	Eastover Rd	Wilson	36.17999	-86.24111

ECOREGION REFERENCE STREAMS

SITE #	STATUS	STREAM	USGS HUC	LOCATION	COUNTY	LATITUDE	LONGITUDE
ECO73A01	Active	Cold Creek	08010100 Mississippi	RM 14.4, U/S Long Hole Rd	Lauderdale	35.7425	-89.6994
ECO73A02	Active	Middle Fork Forked Deer	08010100 Mississippi	RM 3.3, 0.5 miles upstream Watkins Rd	Lauderdale	35.81777	-89.65611
ECO73A03	Active	Cold Creek	08010100 Mississippi	RM 2.3, Approx 1.4 mi u/s Crutcher Lake Rd, U/S Adams Bayou	Lauderdale	35.66305	-89.81222
ECO73A04	Active	Bayou du Chien	08010202 Obion	RM 3.2, Approx 1.5 mi U/S boat ramp on Walnut Log Rd and 0.75 mi U/S last cabin	Lake	36.475	-89.30916
ECO74A06	Active	Sugar Creek	08010100 Mississippi	RM 2.3, U/S Copper Rd	Tipton	35.49944	-89.91914
ECO74A08	Active	Pawpaw Creek	08010202 Obion	RM 3.1, U/S Upper Crossing of Putnam Hill Rd	Obion	36.30527	-89.35666
ECO74B01	Active	Terrapin Creek	08010202 Obion	RM 1.6, Terrapin Creek Rd	Henry	36.48666	-88.48583
ECO74B04	Active	Powell Creek	08010202 Obion	RM 2.2, McClains Levee Rd	Weakley	36.48027	-88.64
ECO74B12	Active	Wolf River	08010210 Wolf	RM 72.7, U/S Yager Rd	Fayette	35.0325	-89.24583

HEADWATER ECOREGION REFERENCE STREAMS

SITE #	STATUS	STREAM	USGS HUC	LOCATION	COUNTY	LATITUDE	LONGITUDE
FECO65E03	Candidate 10-8-08	Dabbs Creek UT to UT	06040001 Tennessee Western Valley-Beech	RM 0.1, Natchez Trace State Park off Todd Trail	Henderson	35.79006	-88.30636
FECO65E04	Candidate 10-8-08	Cubb Creek UT	06040001 Tennessee Western Valley-Beech	RM 0.1, Natchez Trace State Park of Taylor Trail	Henderson	35.78489	-88.26502
FECO65E05	Candidate 8-18-09	Tuscumbia River UT	08010207 Hatchie-Lower	RM 0.6, Big Hill State Park at footbridge on Tuscumbia Trail bend	McNairy	35.05162	-88.74677
FECO65J01	Candidate 12-5-06	Haw Branch	06030005 Tennessee-Pickwick lake	RM 0.9, U/S Pickwick Embayment	Hardin	35.0852	-88.1916

HEADWATER ECOREGION REFERENCE STREAMS

SITE #	STATUS	STREAM	USGS HUC	LOCATION	COUNTY	LATITUDE	LONGITUDE
FECO65J02	Candidate 9-9-08	Horse Creek UT	06040001 Tennessee Western Valley-Beech	RM 0.3, Sugar Camp Hollow	Hardin	35.15521	-88.19176
FECO65J03	Candidate 11-6-08	English Creek	06040001 Tennessee Western Valley-Beech	RM 5.6, South of Firetower Rd near seaton cabin and Ross property line	Hardin	35.15393	-88.17444
FECO66D01	Active	Black Branch	06010103 Watauga	RM 2.0, Above Hwy 231 near Elk Mills TN 195 Black Br Rd-West of US 321	Carter	36.2825	-82.0275
FECO66D06	Active	Tumbling Creek	06010108 Nolichucky	RM 1.5, Just U/S where tumbling Creek ends	Unicoi	36.0180	-82.48194
FECO66D07	Active	Little Stony Creek	06010103 Watauga	RM 2.0, Next to Little Stony Rd and 3.0 miles D/S conf with Goodwin Field Branch	Carter	36.2867	-82.0667
FECO66E01	Candidate 7-15-10	Hell Hollow		TBD			
FECO66G01	Active	Indian Branch	06010204 Little Tennessee	RM 0.1, North River Rd	Monroe	35.33102	-84.06733
FECO66G02	Candidate 4-6-10	Texas Creek	06010107 French Broad-Lower	RM 0.1, Immediately U/S Hwy 321 border of GSMNP	Sevier	35.76229	-83.31250
FECO67F01	Candidate 2-11-10	Clinch River UT	06010205 Clinch-Upper	RM 0.1, Mackey Rd off Chinquapin Rd	Hancock	36.46011	-83.30789
FECO67F02	Candidate 6-9-08	Mill Creek	06010207 Clinch-Lower	RM 1.1, Off Cave Rd	Roane	35.84999	-84.38210
FECO67F03	Candidate 06-10-10	Council Spring	03150101 Conasauga	Red Clay State Park	Bradley	34.99173	-84.94601
FECO67F04	Candidate 4-26-10	Sutton Branch	06010206 Powell	RM 0.1, Off Rob Camp Church Rd	Claiborne	36.558	-83.422
FECO67G02	Candidate 4-19-10	French Broad River UT in Reed Hollow	06010105 French Broad-Upper	RM 0.1, Reed Hollow Tributary to French Broad near Rankin	Cocke	36.05663	-83.19968
FECO67G11	Active	North Prong Fishdam Creek	06010102 Houlston- South Fork	RM 1.6 ,U/S SR 34 (2.0 miles from US 421)	Sullivan	36.5344	-82.0192

HEADWATER ECOREGION REFERENCE STREAMS

SITE #	STATUS	STREAM	USGS HUC	LOCATION	COUNTY	LATITUDE	LONGITUDE
FECO67H01a	Candidate 2-11-10	Taliaferro Branch	06020001 Tennessee	RM 2.4, Firetower Rd	Hamilton	35.16383	-85.01247
FECO67H01b	Candidate 2-11-10	Taliaferro Branch UT	06020001 Tennessee	RM 0.1, Firetower Rd	Hamilton	35.16415	-85.01167
FECO67H04	Active	Blackburn Creek	06020002 Hiwassee	RM 1.8, 0.24 miles U/S Blackburn Hollow Rd	Bradley	35.22472	-84.97055
FECO67H08	Active	Parker Creek	06010104 Holston	RM 1.4, Holston Army Ammunition Plant Property	Hawkins	36.50976	-82.65516
FECO67I01	Candidate 6-10-10	Acre Spring Branch	06020002 Hiwassee	U/S HWY 163	McMinn	35.29789	-84.68172
FECO67I02	Candidate 6-10-10	Oostanaula Creek UT	06020002 Hiwassee	100 yds U/S Clay Hill Road	McMinn	35.37757	-84.63632
FECO67I12	Active	Mill Branch	06010207 Clinch-Lower	RM 1.2, Below the confluence of two tributaries just off Tuskegee Dr	Anderson	35.98833	-84.28888
FECO68A01	Active	Douglas Branch	06010208 Emory	RM 0.1, Barnett Bridge Rd	Morgan	36.14852	-84.77823
FECO68B01	Candidate 6-10-10	Blue Springs	06020004 Hiwassee	RM 0.7, D/S Japser DW plant	Marion	35.08165	-85.63449
FECO68B02	Candidate 6-10-10	Clear Spring Branch	06020004 Hiwassee	RM 1.8, U/S of Valley View Hwy	Marion	35.10273	-85.60249
FECO68C12	Active	Ellis Gap Branch	06020001 Tennessee	RM 0.4, 0.2 miles U/S Mullens Cove Rd in Prentice Cooper State Park	Marion	35.0492	-85.4728
FECO69D01	Active	New RV 1 UT	05130104 Cumberland-South Fork	RM 0.1, U/S Hwy 116	Morgan	36.12090	-84.43214
FECO69D03	Candidate 5-20-09	Bear Branch	06010205 Clinch-Upper	RM 0.1, U/S Hwy 68	Campbell	36.39916	-84.30928
FECO69D04	Candidate 5-20-09	Wheeler Creek UT	05130104 Cumberland-South Fork	RM 0.6, Big Bruce Bridge	Campbell	36.30771	-84.27522
FECO69E01	Candidate 4-29-10	Titus Creek UT	06010205 Clinch-Upper	RM 1.9, U/S of Stinking Creek Rd	Campbell	36.41966	-84.29011
FECO69E02	Candidate 7-13-10	Davis Creek UT	05130101 Clear	Near Woodson Gap on Davis Creek Rd	Campbell	36.47036	-84.02536

HEADWATER ECOREGION REFERENCE STREAMS

SITE #	STATUS	STREAM	USGS HUC	LOCATION	COUNTY	LATITUDE	LONGITUDE
FECO69E03	Candidate 7-13-10	Tackett Creek UT	05130101 Clear	Near Chestnut Ridge	Claiborne	36.4885	-84.8956
FECO71E02	Candidate 6-8-09	Savage Branch	05130206 Red	RM 1.2, U/S Distillery Rd off Hwy 76	Robertson	36.47534	-86.76083
FECO71E03	Candidate 6-8-09	Brush Creek	05130206 Red	RM 9.0, U/S Gause Rd	Robertson	36.4342	-87.06622
FECO71F01	Candidate 6-12-09	Little Swan Creek UT	06040003 Duck-Lower	RM 0.1, Off DP Humphreys Rd	Lewis	35.50053	-87.41683
FECO71F03	Candidate 6-8-09	Ethridge Hollow	06040003 Duck-Lower	RM 0.1, U/S Hwy 230	Humphreys	35.9407	-87.6530
FECO71F04	Candidate 6-8-09	Little Marrowbone Creek UT (Henry)	05130202 Cumberland Lower Cheatham Lake	RM 0.1, U/S Little Marrowbone Rd in Beaman City Park	Davidson	36.27212	-86.9049
FECO71F05	Candidate 6-8-09	Kelley Creek	05130204 Harpeth	RM 2.4, Off Taylor Cemetery Rd	Williamson	35.89778	-87.10004
FECO71F06	Candidate 4-10-11	Mark’s Creek	05130202 Cumberland-Cheatham	HWY 12	Cheatham	36.28544	-87.07753
FECO71G01	Candidate 6-12-09	Flat Creek	05130106 Cumberland- Upper (Cordell Hull)	RM 8.3, Upper Hillman Rd	Overton	36.41239	-85.37442
FECO71G02	Candidate 6-8-09	Long Fork UT	05110002 Barren	RM 0.1, U/S Tanyard Rd	Macon	36.48909	-85.93973
FECO71H03	Candidate 6-8-09	Haws Spring Fork	05130203 Stones	RM 2.7, Off Farm Rd off Jimtown Rd	Cannon	35.761291	-86.08854
FECO71I01	Candidate 5-28-10	Thick Creek UT	06040002 Duck-Upper	RM 1.3, U/S Dowdy Rd	Marshell	35.69278	-86.72745
FECO71I02	Candidate 6-8-09	Young Branch	05130201 Cumberland-Old Hickory Lake	RM 1.6, U/S Hwy 70N	Wilson	36.24031	-86.16099
FECO71I03	Candidate 6-8-09	McKnight Branch UT	05130203 Stones	RM 2.4, U/S Ford off Elrod Mcelroy Rd	Rutherford	35.896901	-86.18094
FECO71I04	Candidate 6-8-09	East Fork Hurricane Creek	05130203 Stones	RM 2.2, Cedar Forest Rd in Cedars of Lebanon State Park	Wilson	36.05598	-86.27829

HEADWATER ECOREGION REFERENCE STREAMS

SITE #	STATUS	STREAM	USGS HUC	LOCATION	COUNTY	LATITUDE	LONGITUDE
FECO71I05	Candidate 6-8-09	West Fork Stones River	05130203 Stones	RM 37.6, U/S Harrison Rd	Rutherford	35.65667	-86.45599
FECO73A01	Candidate 7-13-10		08010202 Obion-North Fork	TBD			
FECO73A03	Candidate 6-18-10	Grassy Lake UT	08010100 Mississippi	RM 0.57, Near Pioneer Spring trail-Meeman State Park	Shelby	35.31831	-90.07124
FECO74A02	Candidate 7-13-09	Running Reelfoot Bayou UT	08010202 Obion	U/S Foothill Rd	Obion	36.3012	-89.39021
FECO74A04	Candidate 6-18-10	Barnishee Bayou UT	08010101 Mississippi	RM 0.89, U/S of Riddick Rd in Meeman Shelby State Park	Shelby	35.35198	-90.04863
FECO74B01	Candidate 2-24-09	North Fork Wolf River UT	08010210 Wolf	RM 0.2, Ames Plantation	Fayette	35.10770	-89.31641
FECO74B02	Candidate 9-18-09	Hatchie River UT	08010208 Hatchie-Lower	RM 2.7, Off Landfill Rd	Haywood	35.54557	-89.30765

Regional Expectations for Individual Habitat Parameters - streams > 2 sq mile drainage

(Values represent 75% of reference condition and may indicate impairment regardless of total habitat score)

ECO	Epifaunal Substrate	Embeddedness	Channel Substrate	Velocity Depth	Pool Variability	Sediment Deposition	Flow Status	Channel Alteration	Riffle Frequency	Channel Sinuosity	Bank Stability (either bank)	Vegetative Protection (either bank)	Riparian Vegetation (either bank)
65a	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
65b	13	NA	8	NA	10	10	11	15	NA	12	6	8	8
65e	14	NA	11	NA	12	14	15	15	NA	13	8	8	8
65i	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
65j	14	14	NA	14	NA	14	14	15	14	NA	8	8	8
66d	15	15	NA	15	NA	14	15	15	15	NA	8	8	8
66e	15	15	NA	15	NA	15	15	15	15	NA	8	8	8
66f	15	15	NA	15	NA	15	15	15	15	NA	8	8	8
66g	15	15	NA	15	NA	15	15	15	15	NA	8	8	8
67f	15	14	NA	15	NA	14	15	15	15	NA	8	8	8
67g	14	13	NA	14	NA	14	14	14	13	NA	7	7	7
67h	13	13	NA	14	NA	11	13	15	14	NA	7	7	8
67i	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
68a Jan-June	14	14	NA	14	NA	15	15	15	14	NA	8	8	8
68a July-Dec	14	14	NA	14	NA	15	14	15	14	NA	8	8	8
68b	13	13	NA	14	NA	12	14	14	14	NA	7	7	7
68c Jan-June	14	15	NA	14	NA	14	15	15	14	NA	7	8	8
68c July-Dec	14	15	NA	14	NA	15	12	15	14	NA	7	8	8
69d Jan-June	14	14	NA	15	NA	14	14	15	15	NA	8	8	8
69d July-Dec	14	13	NA	12	NA	13	12	15	15	NA	8	8	8
69e Jan-June	14	14	NA	15	NA	14	14	15	15	NA	7	8	8
69e July-Dec	14	13	NA	11	NA	12	7	15	14	NA	7	8	8
71e	13	13	NA	14	NA	12	14	14	14	NA	6	7	7
71f	14	14	NA	14	NA	13	14	15	14	NA	7	8	8
71g	14	14	NA	14	NA	14	14	14	14	NA	8	8	8
71h	13	14	NA	14	NA	13	14	14	14	NA	7	7	6
71i high grad.	13	13	NA	13	NA	13	14	14	12	NA	7	7	8
71i low grad.	12	NA	11	NA	13	14	14	14	NA	9	7	6	6
73a	12	NA	10	NA	11	12	15	15	NA	10	6	8	8
74a	11	12	NA	11	NA	9	8	14	13	NA	6	7	7
74b	12	NA	11	NA	11	12	14	15	NA	10	5	8	8

Regional Habitat Expectations for Headwater Streams - ≤ 2 square mile drainage

(Values represent 75% of reference condition and may indicate impairment regardless of total habitat score)

ECO	Epifaunal Substrate	Embeddedness	Channel Substrate	Velocity Depth	Pool Variability	Sediment Deposition	Flow Status	Channel Alteration	Riffle Frequency	Channel Sinuosity	Bank Stability (either bank)	Vegetative Protection (either bank)	Riparian Vegetation (either bank)
65a	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
65b	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
65e	13	NA	8	NA	9	10	11	15	NA	15	6	6	8
65i	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
65j	13	14	NA	11	NA	14	14	15	13	NA	7	7	8
66d	15	15	NA	15	NA	15	15	15	15	NA	8	8	8
66e	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
66f	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
66g	14	12	NA	12	NA	13	15	15	15	NA	8	8	7
67f	14	15	NA	13	NA	13	15	14	15	NA	7	8	8
67g	15	15	NA	10	NA	14	12	15	14	NA	8	8	8
67h	12	14	NA	13	NA	14	14	15	15	NA	8	8	8
67i	12	12	NA	11	NA	12	14	14	13	NA	7	8	7
68a Jan-June	12	14	NA	13	NA	14	15	14	13	NA	7	7	7
68a July-Dec	14	13	NA	12	NA	12	13	15	14	NA	6	6	8
68b	13	11	NA	13	NA	12	12	14	13	NA	8	7	7
68c Jan-June	14	14	NA	13	NA	14	14	15	15	NA	7	8	8
68c July-Dec	14	14	NA	12	NA	12	14	14	14	NA	5	6	8
69d Jan-June	15	13	NA	10	NA	14	15	14	15	NA	8	8	8
69d July-Dec	14	12	NA	11	NA	11	14	14	14	NA	7	7	7
69e Jan-June	14	14	NA	8	NA	14	15	14	15	NA	7	8	7
69e July-Dec	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
71e	14	12	NA	14	NA	11	14	13	14	NA	6	7	7
71f	14	14	NA	11	NA	13	13	15	14	NA	7	8	8
71g	14	12	NA	14	NA	11	13	15	14	NA	7	7	8
71h	13	11	NA	14	NA	11	14	14	14	NA	6	7	7
71i high grad.	14	12	NA	12	NA	11	14	14	14	NA	6	7	7
71i low grad.	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
73a	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
74a	11	11	NA	11	NA	11	7	15	14	NA	6	8	8
74b	12	NA	6	NA	9	13	12	15	NA	12	7	8	8

APPENDIX B

FORMS, FIELD SHEETS AND REPORTS

COUNTY ABBREVIATIONS AND CODE NUMBERS
HABITAT ASSESSMENT FIELD SHEETS
WPC STREAM SURVEY FIELD SHEET
BIORECON FIELD SHEET
BIOLOGICAL SAMPLE REQUEST INCLUDING CHAIN OF CUSTODY FORM
MACROINVERTEBRATE ASSESSMENT REPORT
EXOTIC PLANTS IN TENNESSEE

COUNTIES – Abbreviations and Code Numbers

COUNTY NAME	WPC CO ABBR	TN CO NO	NATIONAL TN FIPS	COUNTY NAME	WPC CO ABBR	TN CO NO	NATIONAL TN FIPS
ANDERSON	**AN**	01	001	LAUDERDALE	**LE**	49	097
BEDFORD	**BE**	02	003	LAWRENCE	**LW**	50	099
BENTON	**BN**	03	005	LEWIS	**LS**	51	101
BLEDSOE	**BL**	04	007	LINCOLN	**LI**	52	103
BLOUNT	**BT**	05	009	LOUDON	**LO**	53	105
BRADLEY	**BR**	06	011	MCMINN	**MM**	54	107
CAMPBELL	**CA**	07	013	MCNAIRY	**MC**	55	109
CANNON	**CN**	08	015	MACON	**MA**	56	111
CARROLL	**CR**	09	017	MADISON	**MN**	57	113
CARTER	**CT**	10	019	MARION	**MI**	58	115
CHEATHAM	**CH**	11	021	MARSHALL	**ML**	59	117
CHESTER	**CS**	12	023	MAURY	**MY**	60	119
CLAIBORNE	**CL**	13	025	MEIGS	**ME**	61	121
CLAY	**CY**	14	027	MONROE	**MO**	62	123
COCKE	**CO**	15	029	MONTGOMERY	**MT**	63	125
COFFEE	**CE**	16	031	MOORE	**MR**	64	127
CROCKETT	**CK**	17	033	MORGAN	**MG**	65	129
CUMBERLAND	**CU**	18	035	OBION	**OB**	66	131
DAVIDSON	**DA**	19	037	OVERTON	**OV**	67	133
DECATUR	**DE**	20	039	PERRY	**PE**	68	135
DE KALB	**DB**	21	041	PICKETT	**PI**	69	137
DICKSON	**DI**	22	043	POLK	**PO**	70	139
DYER	**DY**	23	045	PUTNAM	**PU**	71	141
FAYETTE	**FA**	24	047	RHEA	**RH**	72	143
FENTRESS	**FE**	25	049	ROANE	**RO**	73	145
FRANKLIN	**FR**	26	051	ROBERTSON	**RN**	74	147
GIBSON	**GI**	27	053	RUTHERFORD	**RU**	75	149
GILES	**GS**	28	055	SCOTT	**SC**	76	151
GRAINGER	**GR**	29	057	SEQUATCHIE	**SE**	77	153
GREENE	**GE**	30	059	SEVIER	**SV**	78	155
GRUNDY	**GY**	31	061	SHELBY	**SH**	79	157
HAMBLEN	**HA**	32	063	SMITH	**SM**	80	159
HAMILTON	**HM**	33	065	STEWART	**ST**	81	161
HANCOCK	**HK**	34	067	SULLIVAN	**SU**	82	163
HARDEMAN	**HR**	35	069	SUMNER	**SR**	83	165
HARDIN	**HD**	36	071	TIPTON	**TI**	84	167
HAWKINS	**HS**	37	073	TROUSDALE	**TR**	85	169

COUNTY NAME	WPC CO ABBR	TN CO NO	NATIONAL TN FIPS	COUNTY NAME	WPC CO ABBR	TN CO NO	NATIONAL TN FIPS
HAYWOOD	**HY**	38	075	UNICOI	**UC**	86	171
HENDERSON	**HE**	39	077	UNION	**UN**	87	173
HENRY	**HN**	40	079	VAN BUREN	**VA**	88	175
HICKMAN	**HI**	41	081	WARREN	**WA**	89	177
HOUSTON	**HO**	42	083	WASHINGTON	**WN**	90	179
HUMPHREYS	**HU**	43	085	WAYNE	**WE**	91	181
JACKSON	**JA**	44	087	WEAKLEY	**WY**	92	183
JEFFERSON	**JE**	45	089	WHITE	**WH**	93	185
JOHNSON	**JO**	46	091	WILLIAMSON	**WI**	94	187
KNOX	**KN**	47	093	WILSON	**WS**	95	189
LAKE	**LA**	48	095				

HABITAT ASSESSMENT FIELD SHEET- MODERATE TO HIGH GRADIENT STREAMS (FRONT)
(See Protocol E for detailed descriptions and rank information)

STATION ID:	HABITAT ASSESSED BY:
STREAM NAME:	DATE: TIME:
STATION LOCATION:	ECOREGION: QC: Consensus Duplicate
WBID/HUC: GROUP:	ASSOCIATED LOG #:

	Optimal	Suboptimal	Marginal	Poor
1. Epifaunal Substrate/ Available Cover	Over 70% of stream reach has natural stable habitat suitable for colonization by fish and/or macroinvertebrates. Four or more productive habitats are present.	Natural stable habitat covers 40-70% of stream reach. Three or more productive habitats present. (If near 70% and more than 3 go to optimal.)	Natural stable habitat covers 20 -40% of stream reach or only 1-2 productive habitats present. (If near 40% and more than 2 go to suboptimal.)	Less than 20% stable habitat; lack of habitat is obvious; substrate unstable or lacking.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Comments				
2.Embeddedness of Riffles	Gravel, cobble, and boulders 0-25% surrounded by fine sediment. Layering of cobble provides diversity of niche space. If near 25% drop to suboptimal if riffle not layered cobble.	Gravel, cobble and boulders 25-50% surrounded by fine sediment. Niches in bottom layers of cobble compromised. If near 50% & riffles not layered cobble drop to marginal.	Gravel, cobble, and boulder s are 50-75% surrounded by fine sediment. Niche space in middle layers of cobble is starting to fill with fine sediment.	Gravel, cobble, and boulders are more than 75% surrounded by fine sediment. Niche space is reduced to a single layer or is absent.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Comments				
3. Velocity/ Depth Regime	All four velocity/depth regimes present (slow-deep, slow-shallow, fast-deep, fast-shallow).	Only 3 of the 4 regimes present (if fast-shallow is missing score lower). If slow-deep missing score 15.	Only 2 of the 4 habitat regimes present (if fast-shallow or slow-shallow are missing, score low).	Dominated by 1 velocity/depth regime. Others regimes too small or infrequent to support aquatic populations.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Comments				
4. Sediment Deposition	Sediment deposition affects less than 5% of stream bottom in quiet areas. New deposition on islands and point bars is absent or minimal.	Sediment deposition affects 5-30% of stream bottom. Slight deposition in pool or slow areas. Some new deposition on islands and point bars. Move to marginal if build-up approaches 30%.	Sediment deposition affects 30-50% of stream bottom. Sediment deposits at obstruction, constrictions and bends. Moderate pool deposition.	Heavy deposits of fine material, increased bar development; more than 50% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Comments				
5. Channel Flow Status. .	Water reaches base of both lower banks and streambed is covered by water throughout reach. Minimal productive habitat is exposed.	Water covers > 75% of streambed or 25% of productive habitat is exposed.	Water covers 25-75% of streambed and/or productive habitat is mostly exposed.	Very little water in channel and mostly present as standing pools. Little or no productive habitat due to lack of water.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Comments				

HABITAT ASSESSMENT FIELD SHEET- MODERATE TO HIGH GRADIENT STREAMS (BACK)

Station ID________________________________ Date______________ Initials______________

	Optimal	Suboptimal	Marginal	Poor
6. Channel Alteration	Channelization, dredging rock removal or 4-wheel activity (past or present) absent or minimal; natural meander pattern. NO artificial structures in reach. Upstream or downstream structures do not affect reach.	Channelization, dredging or 4-wheel activity up to 40%. Channel has stabilized. If larger reach, channelization is historic and stable. Artificial structures in or out of reach do not affect natural flow patterns.	Channelization, dredging or 4-wheel activity 40-80% (or less that has not stabilized.) Artificial structures in or out of reach may have slight affect.	Over 80% of reach channelized, dredged or affected by 4-wheelers. Instream habitat greatly altered or removed. Artificial structures have greatly affected flow pattern.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Comments				
7. Frequency of re-oxygenation zones. Use frequency of riffle or bends for category. Rank by quality.	Occurrence of re-oxygenation zones relatively frequent; ratio of distance between areas divided by average stream width <7:1.	Occurrence of re-oxygenation zones infrequent; distance between areas divided by average stream width is 7 - 15.	Occasional re-oxygenation area. The distance between areas divided by average stream width is over 15 and up to 25.	Generally all flat water or flat bedrock; little opportunity for re-oxygenation. Distance between areas divided by average stream width >25.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Comments				
8. Bank Stability (score each bank) Determine left or right side by facing downstream.	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems <5% of bank affected.	Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion. If approaching 30% score marginal if banks steep.	Moderately unstable; 30-60 % of bank in reach has areas of erosion; high erosion potential during floods, If approaching 60% score poor if banks steep.	Unstable; many eroded area; raw areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.
SCORE____(LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE____(RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
Comments				
9. Vegetative Protective (score each bank) includes vegetation from top of bank to base of bank. Determine left or right side by facing downstream	More than 90% of the bank covered by undisturbed vegetation. All 4 classes (mature trees, understory trees, shrubs, groundcover) are represented and allowed to grow naturally. All plants are native.	70-90% of the bank covered by undisturbed vegetation. One class may not be well represented. Disruption evident but not effecting full plant growth. Non-natives are rare (< 30%)	50-70% of the bank covered by undisturbed vegetation. Two classes of vegetation may not be well represented. Non-native vegetation may be common (30-50%).	Less than 50% of the bank covered by undisturbed vegetation or more than 2 classes are not well represented or most vegetation has been cropped. Non-native vegetation may dominate (> 50%)
SCORE____(LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE____(RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
Comments				
10. Riparian Vegetative Zone Width (score each bank.) Zone begins at top of bank.	Average width of riparian zone > 18 meters. Unpaved footpaths may score 9 if run-off potential is negligible.	Average width of riparian zone 12-18 meters. Score high if areas < 18 meters are small or are minimally disturbed.	Average width of riparian zone 6-11 meters. Score high if areas less than 12 meters are small or are minimally disturbed.	Average width of riparian zone <6 meters. Score high if areas less than 6 meters are small or are minimally disturbed.
SCORE____(LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE____(RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
Comments				

Total Score ________ Comparison to Ecoregion Guidelines (circle): ABOVE or BELOW

If score is below guidelines , result of (circle): Natural Conditions or Human Disturbance

Describe

HABITAT ASSESSMENT FIELD SHEET- LOW GRADIENT STREAMS (FRONT)
(See Protocol E for detailed descriptions and rank information)

STATION ID:	HABITAT ASSESSED BY:
STREAM NAME:	DATE: TIME:
STATION LOCATION:	ECOREGION: QC: Consensus Duplicate
WBID/HUC: GROUP:	ASSOCIATED LOG #:

	Optimal	**Suboptimal**	**Marginal**	**Poor**
1. Epifaunal Substrate/ Available Cover	Over 50% of reach has natural, stable habitat for colonization by macroinvertebrates and/or fish. Three or more productive habitats are present.	Natural stable habitat covers 30-50% of stream reach or less than three habitats are present.	Natural stable habitat 10-30% of stream reach. Availability less than desirable, substrate frequently disturbed or removed. Habitat diversity is reduced.	Less than 10% stable habitat; lack of habitat is obvious; substrate unstable or lacking.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Comments				
2. Channel Substrate Characterization	Good mixture of substrate materials, with gravel and firm sand prevalent; root mats and submerged vegetation common.	Mixture of soft sand, mud or clay; or substrate is fissured bedrock, some root mats and submerged vegetation present.	All mud, clay, soft sand or fissured bedrock bottom, little or no root mat, no submerged vegetation present.	Hard-pan clay, conglomerate or predominantly flat bedrock; no root mat or submerged vegetation.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Comments				
3. Pool Variability	Even mix of large-shallow, large-deep, small-shallow, small-deep pools present.	Majority of pools are large-deep very few shallow.	Shallow pools much more prevalent than deep pools.	Majority of pools small-shallow or pools absent.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Comments				
4. Sediment Deposition	Sediment deposition affects less than 20% of stream bottom in quiet areas. New deposition on islands and point bars is absent or minimal.	Some new increase in bar formation, mostly from gravel, sand or fine sediment; 20-50% of bottom affected. Slight deposition in pools.	Moderate deposition of fine material on old and new bars, 50-80% of bottom affected; sediment deposits at obstructions, constrictions and bends; moderate deposition of pools.	Heavy deposits of fine material, increased bar development; more than 80% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Comments				
5. Channel Flow Status. If water backed up by obstructions (beaver dam, log jams, bedrock during low flow) move assessment reach above or below affected area or consider postponing sampling until accurate assessment of stream can be achieved.	Water reaches base of both lower banks throughout reach. Streambed is covered. Minimal productive habitat is exposed.	Water covers > 75% of streambed and/or < 25% of productive habitat is exposed.	Water covers 25-75% of streambed and/or stable habitat is mostly exposed.	Very little water in channel and mostly present as standing pools. Little or no productive habitat due to lack of water.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Comments				

HABITAT ASSESSMENT FIELD SHEET- LOW GRADIENT STREAMS (BACK)

Station ID________________________________ Date______________ Initials_______________

	Optimal	**Suboptimal**	**Marginal**	**Poor**
6. Channel Alteration	Channelization, dredging or 4-wheel activity absent or minimal; natural meander pattern. NO artificial structures in reach. Upstream or downstream structures do not affect reach.	Channelization, dredging or 4-wheel activity up to 40%. Channel has stabilized. If larger reach, channelization is historic and stable. Artificial structures in or out of reach do not affect natural flow patterns.	Channelization, dredging or 4-wheel activity 40-80% (or less that has not stabilized.) Artificial structures in or out of reach may have slight affect.	Over 80% of reach channelized, dredged or affected by 4-wheelers. Instream habitat greatly altered or removed. Artificial structures may have greatly affected flow pattern.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Comments				
7. Channel Sinuosity (Entire meander sequence not limited to sampling reach)	The bends in the stream increase the stream length 3-4 times longer than if it was in a straight line.	The bends in the stream increase the stream length 2-3 times longer than if it was in a straight line.	The bends in the stream increase the stream length 1 to 2 times longer than if it was in a straight line.	Channel straight; waterway has been channelized for a long distance.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
Comments				
8. Bank Stability (score each bank) Determine left or right side by facing downstream.	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems <5% of bank affected.	Moderately stable; infrequent, small areas of erosion o 5-30% of bank eroded. If approaching 30% score marginal if banks steep.	Moderately unstable; 30-60 % of bank in reach has areas of erosion; high erosion potential during floods, If approaching 60% score poor if banks steep.	Unstable; many eroded area; raw areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.
SCORE____(LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE____(RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
Comments				
9. Vegetative Protective (score each bank) includes vegetation from top of bank to base of bank. Determine left or right side by facing downstream	More than 90% of the bank covered by undisturbed vegetation. All 4 classes (mature trees, understory trees, shrubs, groundcover) are represented and allowed to grow naturally. All plants are native.	70-90% of the bank covered by undisturbed vegetation. One class may not be well represented. Disruption evident but not effecting full plant growth. Non-natives are rare (< 30%)	50-70% of the bank covered by undisturbed vegetation. Two classes of vegetation may not be well represented. Non-native vegetation may be common (30-50%).	Less than 50% of the bank covered by undisturbed vegetation or more than 2 classes are not well represented or most vegetation has been cropped. Non-native vegetation may dominate (> 50%)
SCORE____(LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE____(RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
Comments				
10. Riparian Vegetative Zone Width (score each bank.) Zone begins at top of bank.	Average width of riparian zone > 18 meters. Unpaved footpaths may score 9 if run-off potential is negligible.	Average width of riparian zone 12-18 meters. Score high if areas < 18 meters are small or are minimally disturbed.	Average width of riparian zone 6-11 meters. Score high if areas less than 12 meters are small or are minimally disturbed.	Average width of riparian zone <6 meters. Score high if areas less than 6 meters are small or are minimally disturbed.
SCORE____(LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE____(RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
Comments				

Total Score ________ Comparison to Ecoregion Guidelines (circle): ABOVE or BELOW

If score below guidelines, result of (circle): Natural Conditions or Human Disturbance

Describe

WPC STREAM SURVEY FIELD SHEET (Front)

STREAM SURVEY INFORMATION		
Station ID:	Assessors:	
Stream Name:	Date:	Time:
Station Location:	Stream Order:	RM:
County:	Drainage Area (sq mi):	Watershed Group #
WBID/HUC:	Ecoregion:	U/S Eco:
Latitude DEC/DEG:	TOPO:	Gaz. Page:
Longitude DEC/DEG:	Drainage (Basin)	

PROJECT/PURPOSE (circle): Watershed 303(d) Antideg Reference Other (describe)____________

SAMPLES COLLECTED			
Biorecon EFO Log #	___________________________	Periphyton EFO Log #	___________________________
SQKICK EFO Log #	___________________________	Fish EFO Log #	___________________________
SQBANK EFO Log #	___________________________	Other	Log # ___________________________

CHEM/BACTI (circle): None Routine Nutrients Metals Bacti Other _______________________

FIELD MEASUREMENTS	**Meters Used:**				
pH (su)			Dissolved Oxygen (ppm)		
Conductivity (umhos)					
Temperature (^{o}C)					

Meter problems/comments: ____

Previous 48 hrs precipitation: Unknown None Slight Moderate Heavy Flooding

Ambient Weather: Sunny Cloudy Breezy Rain Snow Air temp (^{o}F):___________

WATERSHED CHARACTERISTICS **Approx. % of Watershed Observed:**

Upstream surrounding land use (estimated %):

Pasture		Residential		Industry			
Crops		Commercial		Mining			
Forest		Urban		Impoundment			

PHYSICAL STREAM CHARACTERISTICS **Approx Length of Stream Assessed (m):**

Surrounding land use (estimated %):

	RDB	LDB		RDB	LDB		RDB	LDB	**OTHERS**	RDB	LDB
Pasture			Residential			Industry					
Crops			Commercial			Mining					
Forest			Urban			Wetland					

Observed Human Disturbance to Stream: S (slight) M (moderate) H (high) Blank = not observed

ATV/OHV		Construction		Livestock		Residential	
Industrial		Impoundment		STP/WWTP		Riparian Loss	
Logging		Row Crop		Mining		Water withdrawal	
Urban:		Road/Hwy		Dredging			
Other (describe):							

% Canopy Cover: Estimated reach average: Open (0-10) Partly Shaded (11-45) Mostly Shaded (46-80) Shaded (> 80)

Measured mid reach: _______ U/S _______ D/S _______LB _______ RB _________Total/384*100

Sediment Deposits: None Slight Moderate High Excessive Blanket

Sediment Type: Sludge Mud Sand Silt None Other ____

Turbidity: Clear Slight Moderate High Opaque **Color**____

Surface Sheen/foam: Bacteria Nutrient Surfactant Other____

Algae Present? None Slight Moderate High Choking **Type**: Diatom Green Filamentous Blue-green

Comments:

WPC STREAM SURVEY FIELD SHEET (Back)

Station ID **Date** **Assessors**

	Riffle	**Run**	**Pool**
Depth (m)			
Width (m)			
Reach Length (m)			

Staff Gauge/Bench Ht	
Flow (cfs)	
High Water Mark (m)	
Bank Height (m)	

Flow Conditions: Dry Isolated Pools Low Moderate High Bankfull Flooding Other __________

Gradient (sample reach): Flat Low Moderate High Cascade Other ___________________________

Size (stream width): V. small (< 1.5m) Small (1-5.3 m) Med. (3-10 m) Large (10-25 m) V. Lrge (> 25m)

Substrate Percent (visual estimates):

	Riffle	**Run**	**Pool**		**Riffle**	**Run**	**Pool**
Boulder (> 10")				Clay (Slick)			
Cobble (2.5-10")				Silt			
Gravel (0.1-2.5")				Detritus (CPOM)			
Bedrock				Muck-Mud (FPOM)			
Sand (Gritty)				Marl (Shell frags.)			

Field Based Assessment	**Info from other field sheets (optional)**
Biorecon Score if Applicable __________ **Indicate level:** Family Genus	**BR TR**____ **EPT** ____ **INTOL**____
If SQSH not collected does benthic community appear impaired? Yes No	**Habitat Score HG**_______**LG**______

Describe basis for determination including possible sources of impairment: ___________________________________

Additional Stream Information

Photos? Yes No **ID and Description** ____

Stream Sketch: (include flow direction, reach distance, distance from bridge, nearest road, sampling points, tribs, outfalls, livestock access, riparian area, potential impacts, etc. Use additional sheet if needed).

BIORECON FIELD SHEET: Revised 7-1-2011

STATION ID:	ASSESSORS:
STREAM NAME:	DATE: TIME:
STATION LOCATION:	ECOREGION: U/S ECOREGION: ORDER: DRAINAGE AREA:
HUC: GROUP:	BIORECON Log #:
QA/QC (Circle): Duplicate Sample Pick Efficiency Taxonomy	VOUCHER VERIFCATION: DATE: INITIALS:

Habitats Sampled:	Riffle	Run Rock	Pool Rock	Macrophytes	Woody Debris	Leaf Packs	Sediment	Undercut bank	Tree Root			
Percent habitat:	%	%	%	%	%	%	%	%	%	%	%	%
# Jabs per habitat:												

Indicate estimated abundance (EA): 1 = rare (1-3 organisms) 2= common (4-9) organisms 3 = abundant (10-49) 4 = dominate (>50 organisms)

TAXA	Field	Voucher
Ephemeroptera		
Plecoptera		
Tricoptera		

TAXA	Field	Voucher
Oligochaeta		
Amphipoda		
Decapoda - Cambaridae		
Isopoda – Asellidae		
Acarina		
Odonata		
Anisoptera		
Zygoptera		
Hemiptera		
Megaloptera		
Coleoptera		

TAXA	Field	Voucher
Diptera		
Chironomidae		
Red		
Non-Red		
Tanypodinae		
Athericidae		
Blephaceridae		
Ceratopogonidae		
Empididae		
Simulidae		
Tipulidae		
Mollusca		
Corbiculidae		
Spaheriidae		
Pleuroceridae		
Physidae		

	Family		**Genus**	
	Value	**Score**	**Value**	**Score**
Taxa Richness				
EPT Richness				
Intolerant Taxa				
Total Score	NA		NA	

Comments:

STATE OF ‌NESSEE - ENVIRONMENTAL LABORATORIES

Biological Analysis

Please Print Legibly

**Schedule must be arranged in advance for all tests (615) 262-6327

Project/Site No.	**Screening Bioassays**	**Chronic Bioassays**	Branch Lab Number
Project Name	(Cannot be used for permitting)	Chronic Cd	**Chain of Custody (sign full name)**
Station No. County	48 hr Static Screening Cd	Log Number	1. Collected by
Description	Log Number	LC50 @ 24 hrs	Date Time
Stream Mile Depth	LC50 @ 24 hrs	LC50 @ 48 hrs	Delivered to
Collection Date Time	LC50 @ 48 hrs	LC50 @ 72 hrs	Date Time
Sampler's name (Print)	48 hr Static Screening Pp	LC50 @ 96 hrs	2. Received by
Sampling Agency	Log Number	Survival	Date Time
Billing Code	LC50 @ 24 hrs	NOAEC	Delivered to
If Priority, Date Needed	LC50 @ 48 hrs	LOAEC	Date Time
Send Report to		Reproduction	3. Received by
	Acute Bioassays	NOAEC	Date Time
	48 hr Static Definitive Cd	LOAEC	Delivered to
Contact Hazard	Log Number	IC25	Date Time
Date Reported By	LC50 @ 24 hrs	Chronic Pp	4. Rec'd in Lab by
Reviewed By	LC50 @ 48 hrs	Log Number	Date Time
Reviewed by	NOAEC	LC50 @ 24 hrs	Logged in by
BIOLOGICAL SURVEYS	LOAEC	LC50 @ 48 hrs	Date Time
Macroinvertebrate Recon	48 hr Static Definitive Pp	LC50 @ 72 hrs	**Additional Information**
Rapid Bioassessment (State SOP)	Log Number	LC50 @ 96 hrs	1. Approx. volume of sample
Intensive Survey - Surber	LC50 @ 24 hrs	LC50 @ 120 hrs	2. Nearest town or city
Intensive Survey - Dendy	LC50 @ 48 hrs	LC50 @ 144 hrs	
Fish Population Recon	NOAEC	LC50 @ 168 hrs	3. Others present at collection
Fish Population Intensive	LOAEC	Survival	
Fish Tissue Collection	96 hr Static Definitive Cd	NOAEC	4. Number of other samples collected at same
Chlorophyll Analysis	Log Number	LOAEC	time at this point
Log Number	LC50 @ 24 hrs	Growth	
Chlorophyll a	LC50 @ 48 hrs	NOAEC	5. Field collection procedure, handling and/or
Pheophyton	LC50 @ 72 hrs	LOAEC	preservation of this sample
SPECIAL STUDIES	LC50 @ 96 hrs	IC25	
(Please Specify)	NOAEC		
	LOAEC	Chlorine Residual	6. Mode of transportation to lab
	96 hr Static Definitive Pp		
	Log Number	Lab Parameters	7. Sample/cooler sealed by
	LC50 @ 24 hrs	pH	
	LC50 @ 48 hrs	Cond.	8. Date sample/cooler sealed
	LC50 @ 72 hrs	D.O.	9. Remarks
	LC50 @ 96 hrs	Temp.	
	NOAEC		
	LOAEC		

PH-3010 (rev 1/96) RDA 1527

MACROINVERTEBRATE ASSESSMENT REPORT

Station ID:		**Date Collected:**
Stream:		**Ecoregion(s)**
Location:		**Project**
HUC/ADB Segment:		**Watershed Group:**
Sampled By:	**ID By**	**Scored By:**

If new station send additional information requested on header of stream survey field sheet to PAS

DRAINAGE AREA (circle one): **≤ 2 square miles** **> 2 square miles**

SAMPLE TYPE (circle one) BIORECON SQBANK SQKICK

BIORECON	LAB LOG #		EFO LOG #	
METRIC	**FAMILY LEVEL**		**GENUS LEVEL**	
	Value	**Score**	**Value**	**Score**
Taxa Richness				
EPT Richness				
Intolerant Taxa				

BR INDEX SCORE: Family __________ Genus ____________ SOP date _____________

COMMENTS :

SQSH	LAB LOG #	EFO LOG #
METRIC	**VALUE**	**SCORE**
Total individuals in subsample		**NA**
Taxa Richness		
EPT Richness		
% EPT-Cheum		
% OC		
NCBI		
% Clingers		
% TNUTOL		

TMI SCORE __________ SOP Date _______________

COMMENTS :

HABITAT ASSESSMENT SCORE _______RR/HIGH GRAD. (or) _______GP/LOW GRAD.

HABITAT GUIDELINES FOR SUBREGION (Circle one) ABOVE BELOW

Exotic plants in Tennessee

Compiled by the Tennessee Exotic Plant Pest Council. More information on these species including links to field guides can be found at: http://www.tneppc.org/ Distribution maps for Tennessee can be found at http://plants.usda.gov/checklist.html.

Growth Form	Scientific Name	Common Name
Trees	*Ailanthus altissima* (Mill.) Swingle	Tree of Heaven
	Albizia julibrissin Durz.	Mimosa
	Paulownia tomentosa (Thunb.) Sieb.&Zucc. *ex* Steud.	Princess tree
	Populus alba L.	White poplar
	Broussonetia papyrifera (L.) L'Her. *ex* Vent.	Paper mulberry
	Melia azedarach L.	Chinaberry
	Pyrus calleryana Decne.	Bradford pear
	Triadica (Sapium) sebiferum (L.) Roxb.	Chinese tallowtree
Shrubs	*Elaeagnus umbellata* Thunb.	Autumn olive
	Elaeagnus pungens Thunb.	Thorny-olive
	Ligustrum sinense Lour.	Chinese privet
	Ligustrum vulgare L.	Common privet
	Lonicera fragrantissima Lindl. & Paxton	January jasmine
	Lonicera maackii (Rupr.) Maxim.	Amur bush honeysuckle
	Lonicera morrowii A. Gray	Morrow's bush honeysuckle
	Lonicera tatarica L.	Tartarian honeysuckle; twinsisters
	Lonicera x bella Zabel	Bush honeysuckle
	Rosa multiflora Thunb.	Multiflora rose
	Spiraea japonica L.f.	Japanese spiraea
	Berberis thunbergii DC	Japanese barberry
	Euonymus alata (Thunb.) Sieb.	Burning bush
	Ligustrum japonicum Thunb.	Japanese privet
	Elaeagnus angustifolia L.	Russian olive
	Alnus glutinosa (L.) Gaertn.	Sticky alder
	Hibiscus syriacus L.	Rose of Sharon

Growth Form	**Scientific Name**	**Common Name**
	Rhodotypos scandens (Thunb.) Makino	Jetbead
	Buddleia davidii Franch.	Butterfly bush
Woody Vines	*Celastrus orbiculata* Thunb.	Asian bittersweet
	Euonymus fortunei (Turcz.) Hand.-Mazz.	Winter creeper
	Hedera helix L.	English ivy
	Lonicera japonica Thunb.	Japanese honeysuckle
	Wisteria sinensis (Sims) DC	Chinese wisteria
	Wisteria floribunda (Willd.) DC	Japanese wisteria
	Vinca minor L.	Common periwinkle
	Rhamnus frangula L.	Alder buckthorn
	Ampelopsis brevipedunculata (Maxim.) Trautv.	Amur peppervine
	Rhamnus cathartica L.	European buckthorn
Woody Perennials	*Mahonia beali* (Fortune) Carriere	Oregon grape
	Nandina domestica Thunb.	Nandina, sacred-bamboo
	Rubus phoenicolasius Maxim.	Wineberry
Herbaceous Vines	*Dioscorea oppositifolia* L.	Air-potato
	Pueraria montana (Lour.) Merr.	Kudzu
	Clematis ternifolia DC	Leatherleaf clematis
	Vicia sativa L.	Garden vetch
	Cardiospermum halicacabum L.	Balloonvine, love-in-a-puff
	Tribulus terrestris L.	Puncturevine
	Polygonum perfoliatum L.	Mile-a-minute
"Stout" Herbs	*Polygonum cuspidatum* Seib. & Zucc.	Japanese knotweed; Japanese bamboo
Herbs	*Alliaria petiolata* (Bieb.) Cavara & Grande	Garlic-mustard
	Lespedeza cuneata (Dum.-Cours.) G. Don	Sericea lespedeza
	Lythrum salicaria L. [all varieties and cultivars]	Purple loosestrife
	Solanum viarum Dunal	Tropical soda apple
	Artemisia vulgaris L.	Mugwort, common wormwood
	Carduus nutans L.	Musk thistle, nodding thistle

Growth Form	**Scientific Name**	**Common Name**
	Centaurea biebersteinii DC	Spotted knapweed
	Cirsium arvense (L.) Scop.	Canada thistle
	Cirsium vulgare (Savi) Ten.	Bull thistle
	Conium maculatum L.	Poison hemlock
	Coronilla varia L.	Crown vetch
	Daucus carota L.	Wild carrot, Queen Anne's-lace
	Dipsacus fullonum L.	Fuller's teasle
	Dipsacus laciniatus L.	Cutleaf teasle
	Hesperis matronalis L.	Dame's rocket
	Lespedeza bicolor Turcz.	Bicolor lespedeza, shrubby bushclover
	Lysimachia nummularia L.	Moneywort, creeping Jenny
	Melilotus albus Medik.	White sweet clover
	Melilotus officinalis (L.) Lam.	Yellow sweet clover
	Murdannia keisak (Hassk.) Hand.-Mazz.	Asian spiderwort
	Polygonum caespitosum Blume	Bunchy knotweed, oriental ladies-thumb
	Torilis arvensis (Huds.) Link	Spreading hedge-parsley
	Tussilago farfara L.	Coltsfoot
	Verbascum thapsus L.	Common mullein
	Xanthium strumarium L.	Common cocklebur
	Allium vineale L.	Field Garlic
	Buglossoides arvense (L.) I.M. Johnston	Corn gromwell
	Centaurea cyanus L.	Bachelor's button, cornflower
	Chrysanthemum leucanthemum L.	Ox-eye daisy
	Chicorium intybus L.	Chicory
	Eschscholzia californica Cham.	California poppy
	Fatoua villosa (Thunb.) Nakai	Hairy crabweed
	Glechoma hederacea L.	Gill-over-the-ground, ground ivy
	Iris pseudoacorus L.	Pale-yellow iris
	Kummerowia stipulacea (Maxim.) Makino	Korean clover
	Kummerowia striata (Thunb.) Schindl.	Japanese clover

Growth Form	**Scientific Name**	**Common Name**
	Ornithogalum umbellatum L.	Star of Bethlehem
	Pastinaca sativa L.	Wild parsnip
	Polygonum persicaria L.	Lady's thumb
	Senna obtusifolia (L.) H.S. Irwin & Barneby	Sicklepod senna
	Tragopogon dubius Scop.	Yellow goat's-beard
	Urtica dioica L.	Stinging nettle
	Xanthium spinosum L.	Spiny cocklebur
	Bupleurum rotundifolium L.	Hound's-ear, hare's-ear
	Cosmos bipinnatus Cav.	Garden cosmos
	Cosmos sulphureus Cav.	Sulphur cosmos
	Echium vulgare L.	Viper's bugloss
	Hypericum perforatum L.	Goatweed, St. John's-wort
	Mentha spicata L.	Spearmint
	Mentha x piperita L.	Peppermint
	Muscari atlanticum Boiss. & Reut.	Grape hyacinth
	Muscari botryoides (L.) Mill.	Common grape hyacinth
	Senecio vulgaris L.	Ragwort
	Setaria verticillata (L.) P. Beauv.	Bur-foxtail
	Solanum dulcamara L.	Bittersweet
	Stachys floridana Shuttlew. *ex* Benth.	Hedge nettle
Grasses	*Microstegium vimineum* (Trin.) A. Camus	Nepalgrass; Japanese grass
	Phragmites australis (Cav.) Trin. *ex* Steud.	Common reed
	Sorghum halepense (L.) Pers.	Johnson grass
	Arthraxon hispidus (Thunb.) Makino	Hairy jointgrass
	Bromus commutatus Schrad.	Meadow brome
	Bromus japonicus Thunb. *ex* Murray	Japanese bromegrass
	Bromus secalinus L.	Rye brome
	Bromus tectorum L.	Thatch bromegrass, cheat grass
	Festuca arundinacea Schreb.	Tall fescue
	Festuca pratensis Huds.	Meadow fescue
	Miscanthus sinensis Andersson	Zebra grass, Chinese silver grass
	Setaria faberi R.A.W. Herrm.	Nodding foxtail-grass

Growth Form	Scientific Name	Common Name
	Setaria italica (L.) P. Beauv.	Foxtail-millet
	Setaria pumila (Poir.) Roem. & Schult.	Yellow foxtail, smooth millet
	Setaria viridis (L.) P. Beauv.	Green millet
	Arundo donax L.	Giant reed, elephant grass
	Bromus catharticus Vahl	Bromegrass, rescue grass
	Bromus inermis Leyss.	Smooth bromegrass
	Agrostis stolonifera L.	Weeping love grass
	Bromus hordeaceus L.	Soft brome
	Bromus sterilis L.	Poverty brome
	Imperata cylindrica (L.) Beauv.	Cogon Grass
	Phalaris canariensis L.	Canary grass
	Rottboellia cochinchinensis (Lour.) Clayton	Itchgrass
Aquatic Plants	*Myriophyllum spicatum* L.	Eurasion water milfoil
	Alternanthera philoxeroides (Mart.) Griseb.	Alligatorweed
	Hydrilla verticillata (L.f.) Royle	Hydrilla, water thyme
	Myriophyllum aquaticum (Vell.) Verdc.	Parrot's feather, water milfoil
	Rorippa nasturtium-aquaticum (L.) Hayek	Watercress
	Potamogeton crispus L.	Curly pondweed
	Egeria densa Planch.	Brazilian elodea, Brazilian water-weed
	Najas minor All.	Water nymph
	Salvinia molesta Mitchell	Aquarium water-moss

APPENDIX C

BIOMETRIC INFORMATION

INTOLERANT MACROINVERTEBRATE FAMILIES FOR BIORECONS
TENNESSEE TAXA LIST INCLUDING NCBI SCORES, INTOLERANT TAXA LIST, CLINGER LIST AND VERIFICATION STATUS

Intolerant Macroinvertebrate Families for Biorecons
(Based on average genus NCBI scores for Tennessee taxa within families)

Ephemeroptera
- Ameletidae
- Ephemerellidae
- Leptophlebiidae
- Neoephemeridae
- Polymitarcyidae

Plecoptera
- Capniidae
- Chloroperlidae
- Leuctridae
- Peltoperlidae
- Perlidae
- Perlodidae
- Pteronarcyidae

Trichoptera
- Brachycentridae
- Calamoceratidae
- Glossosomatidae
- Goeridae
- Helicopsychidae
- Lepidostomatidae
- Limnephilidae
- Odontoceridae
- Philopotamidae
- Rhyacophilidae
- Sericostomatidae
- Uenoidae

Coleoptera
- Psephenidae

Diptera
- Athericidae
- Blephariceridae
- Dixidae
- Sciomyzidae

Tennessee Taxa List 2011

Includes NCBI scores, intolerant taxa, list, clinger list and verification status

Order	Family	Genus	NCBI	Intolerant (0.00-3.00)	Clinger	Verification Status
Acarina	Acarina	Acarina	5.53			Verified
Aelosomatida	Aeolosomatidae	Und. Spp.	4			NA
Aeolosomatida	Aeolosomatidae	Aeolosoma	4			Verified
Amphipoda	Crangonyctidae	Crangonyx	7.87			Verified
Amphipoda	Crangonyctidae	Stygobromus				Verified
Amphipoda	Gammaridae	Gammarus	9.1			Verified
Amphipoda	Hyalellidae	Hyalella	7.75			Verified
Amphipoda		Und. Spp.	7.4			NA
Branchiobdellida	Branchiobdellida	Und spp.	6			Verified
Cladocera	Daphniidae	Simocephalus	8			Pending
Coleoptera	Anthicidae	Und. Spp.				NA
Coleoptera	Carabidae	Und. Spp.	4			Pending
Coleoptera	Chrysomelidae	Und. Spp.	4			Pending
Coleoptera	Curculionidae	Onychylis	4			2004 no reference
Coleoptera	Curculionidae	Pnigodes	4			No recent record
Coleoptera	Curculionidae	Tanysphyrus	4			2008 reference pending
Coleoptera	Curculionidae	Und. Spp.	4			Pending
Coleoptera	Dryopidae	Helichus	4.63		X	Verified
Coleoptera	Dryopidae	Pelonomus	4			1996 no reference
Coleoptera	Dytiscidae	Acilius	4			Pending
Coleoptera	Dytiscidae	Agabetes	5.5			2009 reference pending
Coleoptera	Dytiscidae	Agabus	8.9			Pending
Coleoptera	Dytiscidae	Copelatus	10			2009 reference pending
Coleoptera	Dytiscidae	Coptotomus	9.26			Pending
Coleoptera	Dytiscidae	Desmopachria	4			2009 reference pending
Coleoptera	Dytiscidae	Hydaticus	9.1			2001 no reference
Coleoptera	Dytiscidae	Hydroporus	8.62			Pending
Coleoptera	Dytiscidae	Hydrovatus	4.6			2006 reference pending

Order	Family	Genus	NCBI	Intolerant (0.00-3.00)	Clinger	Verification Status
Coleoptera	Dytiscidae	Hygrotus	4			2009 reference pending
Coleoptera	Dytiscidae	Laccodytes	10			2009 reference pending
Coleoptera	Dytiscidae	Laccophilus	10			2009 reference pending
Coleoptera	Dytiscidae	Liodessus	5.5			2007 reference pending
Coleoptera	Dytiscidae	Lioporus	5.5			2009 reference pending
Coleoptera	Dytiscidae	Neoporus	8.52			2009 reference pending
Coleoptera	Dytiscidae	Rhantus	3.61			2008 reference pending
Coleoptera	Dytiscidae	Und. Spp.	5.5			NA
Coleoptera	Dytiscidae	Uvarus	8			Pending
Coleoptera	Elmidae	Ancyronyx	6.49		X	Pending
Coleoptera	Elmidae	Dubiraphia	5.93		X	Pending
Coleoptera	Elmidae	Gonielmis	3.4		X	2004 no reference
Coleoptera	Elmidae	Macronychus	4.58		X	Pending
Coleoptera	Elmidae	Microcylloepus	2.11	X	X	Pending
Coleoptera	Elmidae	Optioservus	2.36	X	X	Pending
Coleoptera	Elmidae	Oulimnius	1.8	X	X	Pending
Coleoptera	Elmidae	Promoresia	2.35	X	X	Pending
Coleoptera	Elmidae	Stenelmis	5.1		X	Pending
Coleoptera	Elmidae	Und. Spp.	6		X	NA
Coleoptera	Gyrinidae	Dineutus	5.54			Pending
Coleoptera	Gyrinidae	Gyrinus	6.17			Verified
Coleoptera	Gyrinidae	Und. Spp.	5.8			NA
Coleoptera	Haliplidae	Haliplus	8.71			Verified
Coleoptera	Haliplidae	Peltodytes	8.73			Pending
Coleoptera	Haliplidae	Und. Spp.	8.7			NA
Coleoptera	Helophoridae	Helophorus	7.57			Verified
Coleoptera	Hydraenidae	Hydraena	4		X	2005 no refereence
Coleoptera	Hydrophilidae	Berosus	8.43			Verified
Coleoptera	Hydrophilidae	Dibolocelus	4.6			No recent record
Coleoptera	Hydrophilidae	Helochares	4			2009 reference pending
Coleoptera	Hydrophilidae	Helocombus	2	X		2001 no reference

Order	Family	Genus	NCBI	Intolerant (0.00-3.00)	Clinger	Verification Status
Coleoptera	Hydrophilidae	Hydrobiomorpha	5			2008 reference pending
Coleoptera	Hydrophilidae	Hydrobius	5			Verified
Coleoptera	Hydrophilidae	Hydrochus	6.55			Pending
Coleoptera	Hydrophilidae	Hydrophilus	2	X		2001 no reference
Coleoptera	Hydrophilidae	Laccobius	7.32			Pending
Coleoptera	Hydrophilidae	Paracymus	4			Pending
Coleoptera	Hydrophilidae	Sperchopsis	6.13		X	Pending
Coleoptera	Hydrophilidae	Tropisternus	9.68			Verified
Coleoptera	Hydrophilidae	Und. Spp.	5			NA
Coleoptera	Hydroptilidae	Enochrus	8.75			Verified
Coleoptera	Noteridae	Hydrocanthus	7.14			2009 reference pending
Coleoptera	Noteridae	Suphisellus	4			2009 reference pending
Coleoptera	Psephenidae	Ectopria	4.16		X	Pending
Coleoptera	Psephenidae	Psephenus	2.35	X	X	Pending
Coleoptera	Psephenidae	Und. Spp.	4		X	NA
Coleoptera	Ptilodactylidae	Anchytarsus	3.64		X	Pending
Coleoptera	Scirtidae	Cyphon	6.8			Verified
Coleoptera	Scirtidae	Elodes	6.8			Pending
Coleoptera	Scirtidae	Prionocyphon	7			Verified
Coleoptera	Scirtidae	Scirtes	6.8			2010 reference pending
Coleoptera	Scirtidae	Und. Spp.	6.8			NA
Coleoptera	Staphylinidae	Bledius	8		X	2001 no reference
Coleoptera	Staphylinidae	Carpelimus	8		X	No recent record
Coleoptera	Staphylinidae	Psephidonus	8			Pending
Coleoptera	Staphylinidae	Stenus	8			Pending
Coleoptera	Staphylinidae	Thinobius	8			Pending
Coleoptera	Staphylinidae	Und. Spp.	8		X	NA
Collembola	Enromobryidae	Sinella	10			1997 no reference
Collembola	Entomobryidae	Entomobrya	10			2010 reference pending
Collembola	Isotomatidae	Hydroistoma	10			2010 reference pending
Collembola	Isotomatidae	Und. Spp.	10			NA

Order	Family	Genus	NCBI	Intolerant (0.00-3.00)	Clinger	Verification Status
Collembola	Isotomidae	Agrenia	8			2009 reference pending
Collembola	Isotomidae	Isotomurus	10			2008 reference pending
Collembola	Onychiuridae	Onychiurus	8			1997 no reference
Collembola	Poduridae	Podura	10			2000 no reference
Collembola	Sminthuridae	Dicyrtoma	10			1996 no reference
Collembola	Sminthuridae	Sminthurides	10			2007 reference pending
Cyclopoida	Cyclopidae	Und. Spp.	8			Verified
Decapoda	Cambaridae	Cambarus	7.62			Verified
Decapoda	Cambaridae	Orconectes	2.6	X		Verified
Decapoda	Cambaridae	Procambarus	9.49			2007 reference pending
Decapoda	Cambaridae	Und. Spp.	7.5			NA
Decapoda	Palaemonidae	Palamontes	7.1			Verified
Diptera	Athericidae	Atherix	2	X		Pending
Diptera	Blephariceridae	Blephariceria	0	X	X	Pending
Diptera	Ceratopogonidae	Alluaudomyia	5.99			Pending
Diptera	Ceratopogonidae	Atrichopogon	6.49			Pending
Diptera	Ceratopogonidae	Bezzia	6			Pending
Diptera	Ceratopogonidae	Bezzia/Palpomyia	6			Pending
Diptera	Ceratopogonidae	Ceratopogon	7.7			Pending
Diptera	Ceratopogonidae	Culicoides	7.7			Pending
Diptera	Ceratopogonidae	Dasyhelea	4			Pending
Diptera	Ceratopogonidae	Forcipomyia	4			2007 reference pending
Diptera	Ceratopogonidae	Mallochohelea	6			Pending
Diptera	Ceratopogonidae	Monohelea	5.7			2008 reference pending
Diptera	Ceratopogonidae	Probezzia	6			Pending
Diptera	Ceratopogonidae	Serromyia	5.7			2010 reference pending
Diptera	Ceratopogonidae	Sphaeromias	5.9			Pending
Diptera	Ceratopogonidae	Stilobezzia	6			Pending
Diptera	Ceratopogonidae	Und. Spp.	5.9			NA
Diptera	Chaoboridae	Mochlonyx	8.5			1998 no reference
Diptera	Chaoboridae	Und. Spp.	8.5			NA

Order	Family	Genus	NCBI	Intolerant (0.00-3.00)	Clinger	Verification Status
Diptera	Chironomidae	Ablabesmyia	7.2			Verified
Diptera	Chironomidae	Acampptocladius				1998 – no reference
Diptera	Chironomidae	Acricotopus				2003 – no reference
Diptera	Chironomidae	Alotanypus				2001 – no reference
Diptera	Chironomidae	Antillocladius				1999 no reference
Diptera	Chironomidae	Apedilum	5.69			2009 reference pending
Diptera	Chironomidae	Apsectrotanypus	0.1	X		Pending
Diptera	Chironomidae	Axarus	2	X		Pending
Diptera	Chironomidae	Brillia	5.18			Verified
Diptera	Chironomidae	Brundiniella	1.71	X		Verified
Diptera	Chironomidae	Cardiocladius	5.87			Verified
Diptera	Chironomidae	Chaetocladius	4			Verified
Diptera	Chironomidae	Chernovkiia	6			1997 no reference
Diptera	Chironomidae	Chironomus	9.63			Verified
Diptera	Chironomidae	Cladopelma	3.49			Verified
Diptera	Chironomidae	Cladotanytarsus	4.09			Verified
Diptera	Chironomidae	Clinotanypus	8.74			Verified
Diptera	Chironomidae	Coelotanypus	8			2004 no reference
Diptera	Chironomidae	Conchapelopia	4.5			Verified
Diptera	Chironomidae	Constempellina	0	X		Verified
Diptera	Chironomidae	Corynoneura	6.01			Verified
Diptera	Chironomidae	Cricotopus	5.78		X	Verified
Diptera	Chironomidae	Cricotopus/Orthocladius	4.86		X	Verified
Diptera	Chironomidae	Cryptochironomus	6.4			Verified
Diptera	Chironomidae	Cryptotendipes	6.19			Verified
Diptera	Chironomidae	Demicryptochironomus	2.12	X		Verified
Diptera	Chironomidae	Diamesa	8.12			Verified
Diptera	Chironomidae	Dicrotendipes	8.1			Verified
Diptera	Chironomidae	Diplocladius	7.41			Verified
Diptera	Chironomidae	Djalmabatista	4			Verified
Diptera	Chironomidae	Doithrix				2005 no reference

Order	Family	Genus	NCBI	Intolerant (0.00-3.00)	Clinger	Verification Status
Diptera	Chironomidae	Einfeldia	7.08			2010 reference pending
Diptera	Chironomidae	Endochironomus	7.79		X	Verified
Diptera	Chironomidae	Endotribelos				2006 reference pending
Diptera	Chironomidae	Epoicocladius	0	X		Verified
Diptera	Chironomidae	Eukiefferiella	3.43			Verified
Diptera	Chironomidae	Euryhapsis				No recent record
Diptera	Chironomidae	Fittkauimyia	5.6			Pending
Diptera	Chironomidae	Glyptotendipes	9.47			Verified
Diptera	Chironomidae	Goeldichironomus	10			Verified
Diptera	Chironomidae	Harnischia	9.07			Verified
Diptera	Chironomidae	Hayesomyia	4.6			2009 reference pending
Diptera	Chironomidae	Heleniella	0	X		Verified
Diptera	Chironomidae	Helopelopia	6.2			Verified
Diptera	Chironomidae	Heterotrissocladius	5.23			Verified
Diptera	Chironomidae	Hydrobaenus	9.54			Verified
Diptera	Chironomidae	Kiefferulus	10			Verified
Diptera	Chironomidae	Krenopelopia				2009 reference pending
Diptera	Chironomidae	Krenosmittia	0	X		Verified
Diptera	Chironomidae	Labrundinia	5.9			Verified
Diptera	Chironomidae	Larsia	9.3			Verified
Diptera	Chironomidae	Limnophyes	7.43			Verified
Diptera	Chironomidae	Lauterborniella				2007 reference pending
Diptera	Chironomidae	Lopescladius	1.67	X		Verified
Diptera	Chironomidae	Meropelopia	2.7	X		Verified
Diptera	Chironomidae	Mesosmittia				2006 reference pending
Diptera	Chironomidae	Metriocnemus				Verified
Diptera	Chironomidae	Micropsectra	1.52	X		Verified
Diptera	Chironomidae	Microtendipes	5.53		X	Verified
Diptera	Chironomidae	Nanocladius	7.07			Verified
Diptera	Chironomidae	Natarsia	9.95			Verified
Diptera	Chironomidae	Neostempellina				Pending
Diptera	Chironomidae	Neozavrelia				2008 reference pending
Diptera	Chironomidae	Nilotanypus	3.9			Verified

Order	Family	Genus	NCBI	Intolerant (0.00-3.00)	Clinger	Verification Status
Diptera	Chironomidae	Nilothauma	5.03			Verified
Diptera	Chironomidae	Omisus	4			2005 no reference
Diptera	Chironomidae	Orthocladius (Euorthocladius)	5.95			Verified
Diptera	Chironomidae	Orthocladius (Symposiocladius)	5.95			Verified
Diptera	Chironomidae	Pagastia	1.77	X		Verified
Diptera	Chironomidae	Parachaetocladius	0	X		Verified
Diptera	Chironomidae	Parachironomus	9.42			Verified
Diptera	Chironomidae	Paracladopelma	5.51			Verified
Diptera	Chironomidae	Paracricotopus	10			Verified
Diptera	Chironomidae	Parakiefferiella	5.4			Verified
Diptera	Chironomidae	Paralauterborniella	4.77		X	Verified
Diptera	Chironomidae	Paramerina	4.29			Verified
Diptera	Chironomidae	Parametriocnemus	3.65			Verified
Diptera	Chironomidae	Paraphaenocladius	3.33			Verified
Diptera	Chironomidae	Parapsectra	1.52	X		Pending
Diptera	Chironomidae	Paratanytarsus	8.45			Verified
Diptera	Chironomidae	Paratendipes	5.11			Verified
Diptera	Chironomidae	Parochlus				2004 no reference
Diptera	Chironomidae	Pentaneura	4.7			Verified
Diptera	Chironomidae	Phaenopsectra	6.5		X	Verified
Diptera	Chironomidae	Phaenopsectra (obedians gp)	6.5		X	Verified
Diptera	Chironomidae	Phaenopsectra (punctipes gp)	6.5		X	Verified
Diptera	Chironomidae	Platysmittia				Verified
Diptera	Chironomidae	Polypedilum	5.69			Verified
Diptera	Chironomidae	Polypedilum (fallax gp)	5.69			Verified
Diptera	Chironomidae	Potthastia	6.4			Verified
Diptera	Chironomidae	Procladius	9.1			Verified
Diptera	Chironomidae	Psectrocladius	3.59			Verified
Diptera	Chironomidae	Pseudochironomus	5.36			Verified
Diptera	Chironomidae	Pseudorthocladius	1.51	X		2009 reference pending
Diptera	Chironomidae	Pseudosmittia	2	X		2009 reference pending

Order	Family	Genus	NCBI	Intolerant (0.00-3.00)	Clinger	Verification Status
Diptera	Chironomidae	Psilometriocnemus	2.8	X		2010 reference pending
Diptera	Chironomidae	Rheocricotopus	7.3			Verified
Diptera	Chironomidae	Rheocricotopus (tuberculatus)	7.3			Verified
Diptera	Chironomidae	Rheopelopia	2	X		Verified
Diptera	Chironomidae	Rheosmittia	7			Verified
Diptera	Chironomidae	Rheotanytarsus	5.89		X	Verified
Diptera	Chironomidae	Robackia	2.95	X		Verified
Diptera	Chironomidae	Saetheria	7.07			Verified
Diptera	Chironomidae	Smittia	2	X		Verified
Diptera	Chironomidae	Stelechomyia	5			Pending
Diptera	Chironomidae	Stempellina	0	X		Verified
Diptera	Chironomidae	Stempellinella	4.62			Verified
Diptera	Chironomidae	Stenochironomus	6.45			Verified
Diptera	Chironomidae	Stictochironomus	6.52			Verified
Diptera	Chironomidae	Stilocladius	0.98	X		Pending
Diptera	Chironomidae	Sublettea	1.6	X		Verified
Diptera	Chironomidae	Symbiocladius	5.4			Verified
Diptera	Chironomidae	Symposiocladius	5.34			Verified
Diptera	Chironomidae	Sympotthastia	5.09			2004 no reference
Diptera	Chironomidae	Synorthocladius	4.36			Verified
Diptera	Chironomidae	Tanypus	9.19			Pending
Diptera	Chironomidae	Tanytarsus	6.76			Verified
Diptera	Chironomidae	Thienemanniella	5.86			Verified
Diptera	Chironomidae	Thienemannimyia	6.2			2010 reference pending
Diptera	Chironomidae	Tribelos	6.31			Verified
Diptera	Chironomidae	Trissopelopia	0	X		2005 no reference
Diptera	Chironomidae	Tvetenia	3.65			Verified
Diptera	Chironomidae	Tvetenia (bavarica gp)	3.65			Verified
Diptera	Chironomidae	Tvetenia (discoloripes gp)	3.65			Verified
Diptera	Chironomidae	Und. Spp.	6.2			NA
Diptera	Chironomidae	Unniella	0	X		Verified
Diptera	Chironomidae	Xenochironomus	7.1			2009 reference pending

Order	Family	Genus	NCBI	Intolerant (0.00-3.00)	Clinger	Verification Status
Diptera	Chironomidae	Xestochironomus	2	X		Verified
Diptera	Chironomidae	Xylotopus	5.99			Verified
Diptera	Chironomidae	Zavrelia	5.3			2010 reference pending
Diptera	Chironomidae	Zavreliella				2009 reference pending
Diptera	Chironomidae	Zavrelimyia	9.11			Verified
Diptera	Culicidae	Aedes	8			2009 reference pending
Diptera	Culicidae	Anopheles	8.58			Pending
Diptera	Culicidae	Culex	10			Pending
Diptera	Culicidae	Und. Spp.	8.1			NA
Diptera	Dixidae	Dixa	2.55	X		Pending
Diptera	Dixidae	Dixella	2.55	X		Pending
Diptera	Dixidae	Und. Spp.	2.55	X		NA
Diptera	Dolichopodidae	Und. Spp.	7			NA
Diptera	Emphydridae	Scatella				Pending
Diptera	Empididae	Chelifera	7.57			Pending
Diptera	Empididae	Clinocera	7.57		X	Tentative
Diptera	Empididae	Hemerodromia	7.57			Confirmed
Diptera	Empididae	Neoplasta	7.57			2009 reference pending
Diptera	Empididae	Trichoclinocera	7.57		X	Pending
Diptera	Empididae	Und spp.	7.6			NA
Diptera	Ephydridae	Notiphila	6			1997 no reference
Diptera	Ephydridae	Scatella	6			Pending
Diptera	Ephydridae	Und. Spp.	6			NA
Diptera	Muscidae	Und. Spp.	8.4			NA
Diptera	Nymphomyiidae	Nymphomyia			X	2004 no reference
Diptera	Phoridae	Dohrniphora				1996 – no reference
Diptera	Phoridae	Und. spp.				Pending
Diptera	Psychodidae	Pericoma/ Telmatoscopus	4.2			Pending
Diptera	Psychodidae	Psychoda	9.64			Pending
Diptera	Psychodidae	Und. Spp.	6.9			NA
Diptera	Ptychopteridae	Bittacomorpha	8			2004 no repherence
Diptera	Sciomyzidae	Und. Spp.	2	X		NA

Order	Family	Genus	NCBI	Intolerant (0.00-3.00)	Clinger	Verification Status
Diptera	Simuliidae	Cnephia	6		X	1996 no reference
Diptera	Simuliidae	Ectemnia	0	X	X	1997 no reference
Diptera	Simuliidae	Prosimulium	4.01		X	Verified
Diptera	Simuliidae	Simulium	4		X	Verified
Diptera	Simuliidae	Und. Spp.	3.5		X	NA
Diptera	Stratiomyidae	Allognosta	5.2			2006 reference pending
Diptera	Stratiomyidae	Caloparyphus	5.2			Verified
Diptera	Stratiomyidae	Myxosargus	6			2009 reference pending
Diptera	Stratiomyidae	Nemotelus	4			2007 reference pending
Diptera	Stratiomyidae	Odontomyia	8			2007 reference pending
Diptera	Stratiomyidae	Oxycera				2009 reference pending
Diptera	Stratiomyidae	Stratiomys	8.08			2009 reference pending
Diptera	Stratiomyidae	Und. Spp.	8			NA
Diptera	Syrphidae	Neoascia	9.7			No recent record
Diptera	Syrphidae	Syrphidae	9.69			Verified
Diptera	Tabanidae	Chlorotabanus	8			Pending
Diptera	Tabanidae	Chrysops	6.73			Pending
Diptera	Tabanidae	Diachlorus	8			Pending
Diptera	Tabanidae	Hybomitra	8			Pending
Diptera	Tabanidae	Tabanus	9.22			2009 reference pending
Diptera	Tanyderidae	Protoplasa	4.33			2006 reference pending
Diptera	Tipulidae	Antocha	4.25		X	Verified
Diptera	Tipulidae	Cryptolabis	4.9			1998 no reference
Diptera	Tipulidae	Dactylolabis	6			2009 reference pending
Diptera	Tipulidae	Dicranota	0	X		Verified
Diptera	Tipulidae	Epiphragma	4.9			Terrestial-verified
Diptera	Tipulidae	Erioptera	4.62			Verified
Diptera	Tipulidae	Gonomyia	6			2006 reference pending
Diptera	Tipulidae	Hexatoma	4.31			Verified

Order	Family	Genus	NCBI	Intolerant (0.00-3.00)	Clinger	Verification Status
Diptera	Tipulidae	Limnophila	3	X		Verified
Diptera	Tipulidae	Limonia	9.64			Verified
Diptera	Tipulidae	Lipsothrix				Verified
Diptera	Tipulidae	Molophilus	6			Verified
Diptera	Tipulidae	Ormosia	6.27			Verified
Diptera	Tipulidae	Paradelphomyia				1996 no reference
Diptera	Tipulidae	Pedicia	2	X		Verified
Diptera	Tipulidae	Pilaria	7			2009 reference pending
Diptera	Tipulidae	Pseudolimnophila	7.22			Pending
Diptera	Tipulidae	Rhabdomastix	8			1996 no reference
Diptera	Tipulidae	Tipula	7.33			Pending
Diptera	Tipulidae	Und. Spp.	4.9			NA
Ephemeroptera	Ameletidae	Ameletus	2.38	X		Verified
Ephemeroptera	Baetidae	Acentrella	3.6			Verified
Ephemeroptera	Baetidae	Acerpenna	3.7			Verified
Ephemeroptera	Baetidae	Baetis	4.51			Verified
Ephemeroptera	Baetidae	Callibaetis	9.80			2010 reference pending
Ephemeroptera	Baetidae	Centroptilum	6.6			Verified
Ephemeroptera	Baetidae	Diphetor	1.2	X		Verified
Ephemeroptera	Baetidae	Fallceon	1.2	X		Verified
Ephemeroptera	Baetidae	Heterocloeon	3.48			Verified
Ephemeroptera	Baetidae	Labiobaetis	6			Verified
Ephemeroptera	Baetidae	Paracloeodes	7.4			Pending
Ephemeroptera	Baetidae	Plauditus	4.51			Verified
Ephemeroptera	Baetidae	Procloeon	6.6			Verified
Epehmeroptera	Baetidae	Pseudocentroptiloides	6			Verified
Ephemeroptera	Baetidae	Pseudocloeon	4			2009 reference pending
Ephemeroptera	Baetidae	Und. Spp.	6.1			NA
Ephemeroptera	Baetiscidae	Baetisca	3.4			Verified
Ephemeroptera	Caenidae	Brachycercus	3.01			Verified
Ephemeroptera	Caenidae	Caenis	7.41			Verified
Ephemeroptera	Ephemerellidae	Attenella	1.56	X	X	No reference
Ephemeroptera	Ephemerellidae	Dannella	3.6		X	Verified
Ephemeroptera	Ephemerellidae	Drunella	0.26	X	X	Verified

Order	Family	Genus	NCBI	Intolerant (0.00-3.00)	Clinger	Verification Status
Ephemeroptera	Ephemerellidae	Ephemerella	2.04	X	X	Verified
Ephemeroptera	Ephemerellidae	Eurylophella	4.34		X	Verified
Ephemeroptera	Ephemerellidae	Serratella	1.57	X	X	Verified
Ephemeroptera	Ephemerellidae	Timpanoga	4.34		X	Verified
Ephemeroptera	Ephemerellidae	Und spp.	1.9	X	X	NA
Ephemeroptera	Ephemeridae	Ephemera	1.12	X		Verified
Ephemeroptera	Ephemeridae	Hexagenia	4.9			Verified
Ephemeroptera	Ephemeridae	Pentagenia	1.8	X		No recent record
Ephemeroptera	Ephemeridae	Und spp.	1.8	X		NA
Ephemeroptera	Heptageniidae	Cinygmula	0	X	X	Verified
Ephemeroptera	Heptageniidae	Epeorus	1.27	X	X	Verified
Ephemeroptera	Heptageniidae	Heptagenia	2.57	X	X	Verified
Ephemeroptera	Heptageniidae	Leucrocuta	2.4	X	X	Verified
Ephemeroptera	Heptageniidae	Maccaffertium	3.15		X	Verified
Ephemeroptera	Heptageniidae	Nixe	0	X	X	1997 no referernce
Ephemeroptera	Heptageniidae	Rhithrogena	0.3	X	X	Verified
Ephemeroptera	Heptageniidae	Stenacron	3.58		X	Verified
Ephemeroptera	Heptageniidae	Stenonema	3.45		X	Verified
Ephemeroptera	Heptageniidae	Und spp.	4		X	NA
Ephemeroptera	Isonychiidae	Isonychia	3.45			Verified
Ephemeroptera	Leptohyphidae	Tricorythodes	5.06			Verified
Ephemeroptera	Leptohyphidae	Und spp.	5.1			NA
Ephemeroptera	Leptophlebiidae	Choroterpes	0	X	X	Verified
Ephemeroptera	Leptophlebiidae	Habrophlebia	0	X		Verified
Ephemeroptera	Leptophlebiidae	Habrophlebiodes	2	X		Verified
Ephemeroptera	Leptophlebiidae	Leptophlebia	6.23			Verified
Ephemeroptera	Leptophlebiidae	Neochoroterpes	2	X		1998 no reference
Ephemeroptera	Leptophlebiidae	Paraleptophlebia	0.94	X		Verified
Ephemeroptera	Leptophlebiidae	Und spp.	1.8	X		NA
Ephemeroptera	Metropodidae	Siphloplecton	3.31			Pending
Ephemeroptera	Neoephemeridae	Neoephemera	0.81	X		Verified
Ephemeroptera	Polymitarcyidae	Ephoron	1.32	X		Verified
Ephemeroptera	Polymitarcyidae	Und spp.	0.7	X		NA
Ephemeroptera	Potamanthidae	Anthopotamus	4			Verified
Ephemeroptera	Siphlonuridae	Siphlonurus	5.81			Verified

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Ephemeroptera	Siphlonuridae	Und spp.	4.1			NA
Gordiida	Gordiidae	Gordius	6			Pending
Haplonemertea	Tertastemmatidae	Prostoma	6.1			Pending
Haplotaxida	Lumbricidae	Haplotaxis	10			Verified
Harpacticoida	Harpacticoida	Und. Spp.	8			NA
Hemiptera	Belostomatidae	Belostoma	9.8			Verified
Hemiptera	Belostomatidae	Lethocerus	4			2000 no reference
Hemiptera	Belostomatidae	Und. Spp.	6.9			NA
Hemiptera	Corixidae	Und. Spp.	9			NA
Hemiptera	Corixidae	Palmacorixa	6			Verified
Hemiptera	Corixidae	Sigara	9.06			Verified
Hemiptera	Corixidae	Trichocorixa	6			Verified
Hemiptera	Gelastocoridae	Gelastocoris				Verified
Hemiptera	Gerridae	Aquarius	5			Verified
Hemiptera	Gerridae	Gerris	6			2009 reference pending
Hemiptera	Gerridae	Limnoporus	6			2009 reference pending
Hemiptera	Gerridae	Metrobates	6			Verified
Hemiptera	Gerridae	Rheumatobates	6			Verified
Hemiptera	Gerridae	Trepobates	6			Verified
Hemiptera	Gerridae	Und. Spp.	6			NA
Hemiptera	Hebridae	Hebrus	6			Verified
Hemiptera	Hebridae	Lipogomphus	6			Verified
Hemiptera	Hydrometridae	Hydrometra	6			Verified
Hemiptera	Mesoveliidae	Mesovelia	6			Verified
Hemiptera	Nepidae	Ranatra	7.82			Verified
Hemiptera	Notonectidae	Bueno	4			Verified
Hemiptera	Notonectidae	Notonecta	8.71			Verified
Hemiptera	Notonectidae	Und. Spp.	6.4			NA
Hemiptera	Pleidae	Neoplea	6			Verified
Hemiptera	Pleidae	Und. Spp.	6			NA
Hemiptera	Saldidae	Pentacora				1998 no reference
Hemiptera	Saldidae	Und. Spp.	10			NA
Hemiptera	Veliidae	Microvelia	6			Verified
Hemiptera	Veliidae	Rhagovelia	6			Verified

Order	Family	Genus	NCBI	Intolerant (0.00-3.00)	Clinger	Verification Status
Hemiptera	Veliidae	Und. Spp.	6			NA
Hydroida	Hydridae	Hydra	6			Pending
Isopoda	Asellidae	Asellus	9.11			Verified
Isopoda	Asellidae	Caecidotea	9.11			Verified
Isopoda	Asellidae	Lirceus	7.85			Verified
Isopoda	Asellidae	Und. Spp.	8			NA
Lepidoptera	Nepticulidae	Und. Spp.				NA
Lepidoptera	Noctuidae	Simyra				Verified
Lepidoptera	Noctuidae	Und. Spp.				NA
Lepidoptera	Pyralidae	Acentria	5			2004 no reference
Lepidoptera	Pyralidae	Crambus	4.6			2007 reference pending
Lepidoptera	Pyralidae	Munroessa/Syncllita	4.3			1998 no referernce
Lepidoptera	Pyralidae	Petrophila	2.09	X	X	Verified
Lepidoptera	Pyralidae	Synclita	5			1997 no reference
Lepidoptera	Pyralidae	Und. Spp.	4.3			NA
Lepidoptera	Tortricidae	Archips				Verified
Limnophila	Ancylidae	Ferrissia	6.55			Verified
Limnophila	Ancylidae	Laevapex	7.49			Verified
Limnophila	Ancylidae	Und. Spp.	7			NA
Limnophila	Lymnaeidae	Lymnaea	6.9			2003 no reference
Limnophila	Lymnaeidae	Pseudosuccinea	7.65			Verified
Limnophila	Lymnaeidae	Und spp.	6.9			NA
Limnophila	Physidae	Physella	8.84			Verified
Limnophila	Planorbidae	Gyraulus	4.23			Pending
Limnophila	Planorbidae	Helisoma	6			Verified
Limnophila	Planorbidae	Micromenetus	6.3			Verified
Limnophila	Planorbidae	Menetus	8.23			2009 reference pending
Limnophila	Planorbidae	Planorbella	6.82			Pending
Limnophila	Planorbidae	Planorbula	6.82			2001 no reference
Limnophila	Planorbidae	Und. Spp.	6.3			NA
Lumbriculida	Lumbriculidae	Eclipidrilus	7.03			2003 no reference
Lumbriculida	Lumbriculidae	Lumbriculus	7.03			Verified
Lumbriculida	Lumbriculidae	Und. Spp.	7.03			NA

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Lymnophila	Limnophila	Stagnicola	8.22			2007 reference pending
Megaloptera	Corydalidae	Chauliodes	8.98		X	Pending
Megaloptera	Corydalidae	Corydalus	5.16		X	Verified
Megaloptera	Corydalidae	Nigronia	5.25		X	Verified
Megaloptera	Corydalidae	Und. Spp	6.5		X	NA
Megaloptera	Sialidae	Sialis	7.17			Verified
Mermithida	Mermithidae	Und. Spp. (nematodes)	6.02			Verified
Mesogastropoda	Hydrobiidae	Hydrobiidae	5.78			Pending
Mesogastropoda	Lymnaeidae	Fossaria	6			Verified
Mesogastropoda	Pleuroceridae	Elimia	2.46	X		Verified
Mesogastropoda	Pleuroceridae	Leptoxis	1.79	X		Verified
Mesogastropoda	Pleuroceridae	Pleurocera	6			Verified
Mesogastropoda	Pleuroceridae	Und. Spp,	3.4			NA
Mesogastropoda	Valvatidae	Valvata	8			1998 no reference
Mesogastropoda	Viviparidae	Campeloma	6.8			Verified
Mesogastropoda	Viviparidae	Viviparus	6			2003 no reference
Mysidacea	Mysidae	Mysis				1998 no reference
Mysidacea	Mysidae	Taphromysis				Verified
Neuroptera	Sisyridae	Sisyra				2009 reference pending
Odonata	Aeshnidae	Basiaeschna	7.35			Verified
Odonata	Aeshnidae	Boyeria	5.97			Verified
Odonata	Aeshnidae	Nasiaeschna	8.14			Verified
Odonata	Aeshnidae	Und. Spp.	5.6			NA
Odonata	Calopterygidae	Calopteryx	7.78			Verified
Odonata	Calopterygidae	Hetaerina	5.61			2007 reference pending
Odonata	Coenagrionidae	Amphiagrion	5			No recent record
Odonata	Coenagrionidae	Argia	8.17			Verified
Odonata	Coenagrionidae	Chromagrion	2	X		2000 no reference
Odonata	Coenagrionidae	Enallagma	8.91			Verified
Odonata	Coenagrionidae	Ischnura	9.52			Verified
Odonata	Coenagrionidae	Und. Spp.	6.1			NA
Odonata	Cordulegastridae	Cordulegaster	5.73			Verified
Odonata	Corduliidae	Didymops	2.36	X		Verified

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Odonata	Corduliidae	Epitheca (Epicordulia)	8.57			Verified
Odonata	Corduliidae	Epitheca (Tetragoneuria)	8.57			Verified
Odonata	Corduliidae	Helocordulia	4.83			Verified
Odonata	Corduliidae	Macromia	6.16			Verified
Odonata	Corduliidae	Neurocordulia	5.03			Verified
Odonata	Corduliidae	Somatochlora	9.15			Verified
Odonata	Corduliidae	Und. Spp.	6.6			NA
Odonata	Gomphidae	Arigomphus				Verified
Odonata	Gomphidae	Dromogomphus	5.92			Verified
Odonata	Gomphidae	Gomphidae	5			Verified
Odonata	Gomphidae	Gomphus	5.8			Verified
Odonata	Gomphidae	Hagenius	3.99			Verified
Odonata	Gomphidae	Lanthus	1.77	X		Verified
Odonata	Gomphidae	Ophiogomphus	5.54			Verified
Odonata	Gomphidae	Progomphus	8.22			Verified
Odonata	Gomphidae	Stylogomphus	4.72			Verified
Odonata	Gomphidae	Stylurus	5.8			Verified
Odonata	Lestidae	Lestes	9.42			2000 no reference
Odonata	Libellulidae	Erythemis	9.72			2003 no reference
Odonata	Libellulidae	Libellula	9.64			2009 reference pending
Odonata	Libellulidae	Nannothemis	9			2000 no reference
Odonata	Libellulidae	Pachydiplax	9.86			2007 reference pending
Odonata	Libellulidae	Perithemis	9.85			Verified
Odonata	Libellulidae	Und. Spp.	6.7			NA
Ostracoda		Podocopa	8			Verified
Plecoptera	Capniidae	Allocapnia	2.52	X	X	Tentative
Plecoptera	Capniidae	Paracapnia	0.12	X		Verified
Plecoptera	Capniidae	Und. Spp.	0.9	X		NA
Plecoptera	Chloroperlidae	Alloperla	1.22	X	X	Verified
Plecoptera	Chloroperlidae	Haploperla	0.98	X	X	Verified
Plecoptera	Chloroperlidae	Rasvena	1	X		Verified
Plecoptera	Chloroperlidae	Sweltsa	0	X	X	Verified

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Plecoptera	Chloroperlidae	Und. Spp.	0.7	X	X	NA
Plecoptera	Leuctridae	Leuctra	0.67	X		Verified
Plecoptera	Leuctridae	Paraleuctra	0.67	X		2005 no reference
Plecoptera	Leuctridae	Und. Spp.	0.2	X		NA
Plecoptera	Leuctridae	Zealeuctra	0	X		Verified
Plecoptera	Nemouridae	Amphinemura	3.33			Verified
Plecoptera	Nemouridae	Nemoura	2	X		2010 reference pending
Plecoptera	Nemouridae	Paranemoura	3.3			Verified
Plecoptera	Nemouridae	Soyedina	0	X		Verified
Plecoptera	Nemouridae	Und. Spp.	1.2	X		NA
Plecoptera	Peltoperlidae	Peltoperla	4.2		X	Verified
Plecoptera	Peltoperlidae	Tallaperla	1.18	X	X	Verified
Plecoptera	Peltoperlidae	Und. Spp.	3	X	X	NA
Plecoptera	Peltoperlidae	Viehoperla	2.7	X	X	Pending
Plecoptera	Perlidae	Acroneuria	1.47	X	X	Verified
Plecoptera	Perlidae	Agnetina	0	X	X	Verified
Plecoptera	Perlidae	Attaneuria	0	X	X	2007 reference pending
Plecoptera	Perlidae	Beloneuria	0	X	X	Tentative
Plecoptera	Perlidae	Eccoptura	3.74		X	Verified
Plecoptera	Perlidae	Hansonoperla				Tentative
Plecoptera	Perlidae	Neoperla	1.49	X	X	Verified
Plecoptera	Perlidae	Paragnetina	1.54	X	X	Verified
Plecoptera	Perlidae	Perlesta	4.7		X	Verified
Plecoptera	Perlidae	Perlinella	0.63	X	X	2007 reference pending
Plecoptera	Perlidae	Und. Spp.	1.5	X	X	NA
Plecoptera	Perlodidae	Clioperla	4.8		X	Verified
Plecoptera	Perlodidae	Cultus	1.57	X	X	2010 reference pending
Plecoptera	Perlodidae	Diploperla	2.06	X	X	Pending
Plecoptera	Perlodidae	Diura	2	X	X	2010 reference pending
Plecoptera	Perlodidae	Helopicus	0.41	X	X	Verified
Plecoptera	Perlodidae	Hydroperla	1.6	X	X	No recent record
Plecoptera	Perlodidae	Isogenoides	0.54	X	X	Verified
Plecoptera	Perlodidae	Isoperla	1.5	X	X	Verified

Order	Family	Genus	NCBI	Intolerant (0.00-3.00)	Clinger	Verification Status
Plecoptera	Perlodidae	Malirekus	1.15	X		Verified
Plecoptera	Perlodidae	Remenus	0.2	X	X	Verified
Plecoptera	Perlodidae	Und. Spp.	1.6	X	X	NA
Plecoptera	Perlodidae	Yugus	0	X	X	Verified
Plecoptera	Pteronarcyidae	Pteronarcys	1.67	X	X	Verified
Plecoptera	Taeniopterygidae	Oemopteryx	1	X		Verified
Plecoptera	Taeniopterygidae	Strophopteryx	2.7	X		Verified
Plecoptera	Taeniopterygidae	Taenionema	2	X		Verified
Plecoptera	Taeniopterygidae	Taeniopteryx	5.37			Tentative
Plecoptera	Taeniopterygidae	Und. Spp.	4			NA
Podocopida	Podocopida	Und. Spp.	8			NA
Rhynchobdellida	Glossiphoniidae	Helobdella	9.1			Pending
Rhynchobdellida	Glossiphoniidae	Mooreobdella	9.43			Pending
Rhynchobdellida	Glossiphoniidae	Placobdella	9			Pending
Rhynchobdellida	Glossiphoniidae	Und. Spp.	9			NA
Trichoptera	Apataniidae	Apatania	0.64	X	X	Verified
Trichoptera	Beraeidae	Beraea				1997 no record
Trichoptera	Brachycentridae	Brachycentrus	2.08	X	X	Verified
Trichoptera	Brachycentridae	Micrasema	0.56	X	X	Verified
Trichoptera	Brachycentridae	Und. Spp.	1	X	X	NA
Trichoptera	Calamoceratidae	Anisocentropus	0.85	X		Verified
Trichoptera	Calamoceratidae	Heteroplectron	3.23			Verified
Trichoptera	Dipseudopsidae	Phylocentropus	6.2			Verified
Trichoptera	Glossosomatidae	Agapetus	0	X	X	Verified
Trichoptera	Glossosomatidae	Glossosoma	1.55	X	X	Verified
Trichoptera	Glossosomatidae	Matrioptila	0	X	X	1999 no reference
Trichoptera	Glossosomatidae	Und. Spp.	1	X	X	NA
Trichoptera	Goeridae	Goera	0.1	X	X	Verified
Trichoptera	Goeridae	Goerita	0	X	X	2007 reference pending
Trichoptera	Goeridae	Und. Spp.	0.05	X	X	NA
Trichoptera	Helicopsychidae	Helicopsyche	0	X	X	Verified
Trichoptera	Hydropsychidae	Arctopsyche	0	X	X	Verified
Trichoptera	Hydropsychidae	Ceratopsyche	3.11		X	Verified
Trichoptera	Hydropsychidae	Cheumatopsyche	6.22		X	Verified
Trichoptera	Hydropsychidae	Diplectrona	2.21	X	X	Verified

Order	Family	Genus	NCBI	Intolerant (0.00-3.00)	Clinger	Verification Status
Trichoptera	Hydropsychidae	Hydropsyche	4.3		X	Verified
Trichoptera	Hydropsychidae	Macrostemum	3.52		X	Verified
Trichoptera	Hydropsychidae	Parapsyche	0	X	X	Verified
Trichoptera	Hydropsychidae	Und. Spp.	4		X	NA
Trichoptera	Hydroptilidae	Agraylea	2	X		2004 no reference
Trichoptera	Hydroptilidae	Hydroptila	6.22		X	Verified
Trichoptera	Hydroptilidae	Leucotrichia	4.06		X	2009 reference pending
Trichoptera	Hydroptilidae	Mayatrichia	0	X	X	Verified
Trichoptera	Hydroptilidae	Neotrichia	0	X	X	2009 reference pending
Trichoptera	Hydroptilidae	Ochrotrichia	3.95		X	Verified
Trichoptera	Hydroptilidae	Orthotrichia	8.29		X	2009 reference pending
Trichoptera	Hydroptilidae	Oxyethira	2.22	X		Verified
Trichoptera	Hydroptilidae	Und. Spp.	4			NA
Trichoptera	Lepidostomatidae	Lepidostoma	0.9	X		Verified
Trichoptera	Lepidostomatidae	Theliopsyche	0	X		2007 reference pending
Trichoptera	Lepidostomatidae	Und. Spp.	0.9	X		NA
Trichoptera	Leptoceridae	Ceraclea	2.01	X		Verified
Trichoptera	Leptoceridae	Mystacides	2.69	X		Verified
Trichoptera	Leptoceridae	Nectopsyche	2.94	X		2009 reference pending
Trichoptera	Leptoceridae	Oecetis	4.7		X	Verified
Trichoptera	Leptoceridae	Setodes	0	X	X	2008 reference pending
Trichoptera	Leptoceridae	Triaenodes	4.46			Verified
Trichoptera	Leptoceridae	Und. Spp.	4			NA
Trichoptera	Limnephilidae	Glyphopsyche	1.00	X		Pending
Trichoptera	Limnephilidae	Hesperophylax	5			No recent record
Trichoptera	Limnephilidae	Hydatophylax	2.17	X		Verified
Trichoptera	Limnephilidae	Ironoquia	7.78			2009 reference pending
Trichoptera	Limnephilidae	Limnephilus	2	X		2001 no reference
Trichoptera	Limnephilidae	Pycnopsyche	2.52	X		Verified
Trichoptera	Limnephilidae	Und. Spp.	2	X		NA

Order	Family	Genus	NCBI	Intolerant (0.00-3.00)	Clinger	Verification Status
Trichoptera	Molannidae	Molanna	4.27			Verified
Trichoptera	Odontoceridae	Psilotreta	0	X		Verified
Trichoptera	Odontoceridae	Und. Spp.	0	X		NA
Trichoptera	Philopotamidae	Chimarra	2.76	X	X	Verified
Trichoptera	Philopotamidae	Dolophilodes	0.81	X	X	Verified
Trichoptera	Philopotamidae	Fumonta	1.4	X		No recent record
Trichoptera	Philopotamidae	Und. Spp.	1.4	X	X	NA
Trichoptera	Philopotamidae	Wormaldia	0.65	X	X	Verified
Trichoptera	Phryganeidae	Ptilostomis	6.37			Verified
Trichoptera	Phryganeidae	Und. Spp.	4			NA
Trichoptera	Polycentropodidae	Cernotina	4		X	2007 reference pending
Trichoptera	Polycentropodidae	Cyrnellus	7.34		X	Pending
Trichoptera	Polycentropodidae	Neureclipsis	4.19		X	Verified
Trichoptera	Polycentropodidae	Paranyctiophylax	0.9	X	X	Verified
Trichoptera	Polycentropodidae	Polycentropus	3.53		X	Verified
Trichoptera	Polycentropodidae	Und. Spp.	4		X	NA
Trichoptera	Psychomyiidae	Lype	4.05		X	Verified
Trichoptera	Psychomyiidae	Psychomyia	2.44	X	X	Verified
Trichoptera	Psychomyiidae	Und. Spp.	3.2		X	NA
Trichoptera	Rhyacophilidae	Rhyacophila	0.73	X	X	Verified
Trichoptera	Sericostomatidae	Agarodes	0.69	X		Verified
Trichoptera	Sericostomatidae	Fattigia	0.9	X		No recent record
Trichoptera	Uenoidae	Neophylax	2.2	X	X	Verified
Tricladida	Dugesiidae	Cura	4.97			Pending
Tricladida	Dugesiidae	Dugesia	7.23			Pending
Tricladida	Dugesiidae	Girardia	7.23			Pending
Tricladida	Planaridae	Sphalloplana	6.1			Pending
Tricladida	Planaridae	Und. Spp.	6.1			NA
Tubificida	Enchytraeidae	Enchytraeidae	9.84			Verified
Tubificida	Naididae	Bratislavia	6			Verified
Tubificida	Naididae	Chaetogaster	4			Pending

Order	Family	Genus	NCBI	Intolerant (0.00-3.00)	Clinger	Verification Status
Tubificida	Naididae	Dero	10			Verified
Tubificida	Naididae	Haemonais	4			Verified
Tubificida	Naididae	Nais	8.88			Verified
Tubificida	Naididae	Ophidonais	2	X		2007 reference pending
Tubificida	Naididae	Piguetiella				Verified
Tubificida	Naididae	Pristina	9.56			Verified
Tubificida	Naididae	Pristinella	7.74			Verfied
Tubificida	Naididae	Slavina	7.06			Verfied
Tubificida	Naididae	Specaria	4			2008 reference pending
Tubificida	Naididae	Stephensoniana	4			2010 reference pending
Tubificida	Naididae	Stylaria	9.38			Verfied
Tubificida	Naididae	Und. Spp.	6.1			NA
Tubificida	Tubificidae	Aulodrilus	4.63			Verified
Tubificida	Tubificidae	Branchiura	8.28			Pending
Tubificida	Tubificidae	Ilyodrilus	9.26			Verified
Tubificida	Tubificidae	imm. w. cap. cht.	7.11			Verfied
Tubificida	Tubificidae	imm. w/o cap. cht.	9.5			Verified
Tubificida	Tubificidae	Isochaetides	8.6			2006 reference pending
Tubificida	Tubificidae	Limnodrilus	9.5			Verified
Tubificida	Tubificidae	Quistadrilus	3.86			Verfied
Tubificida	Tubificidae	Rhyacodrilus	2	X		Verified
Tubificida	Tubificidae	Spirosperma	5.3			Verfied
Tubificida	Tubificidae	Tubifex	10			2009 reference pending
Unionoida	Unionidae	Alasmidonta				Pending
Unionoida	Unionidae	Plectomerus				Pending
Urnatellida	Urnotellidae	Urnatella				1996 no reference
Veneroida	Corbiculidae	Corbicula	6.12			Verified
Veneroida	Sphaeriidae	Eupera	5.73			Verified
Veneroida	Sphaeriidae	Pisidium	6.48			Verified
Veneroida	Sphaeriidae	Und. Spp.	6.6			NA
Veneroida	Sphaeriidae	Sphaerium	7.58			Verified

APPENDIX D

TAXONOMIC INFORMATION

GENUS LEVEL TAXONOMIC KEYS
CRITERIA FOR TAXONOMIC EXPERTS
TAXONOMIC SPECIALISTS FOR REFERENCE VERIFICATION

GENUS LEVEL TAXONOMIC KEYS
(Primary Key for each group is listed first)

TURBELLARIA

Kenk, R. 1972. *Freshwater Planarians (Turbellaria) of North America*. EPA-WPCRS, 18050/ELD 02/72. Supt. Doc. No. 5501-0365. Washington, D.C.

MOLLUSCA

Gastropoda

Burch, J.B. 1989. North American Freshwater Snails. Malacological Publications, Hamburg, MI.

Burch, J.B. 1982. *Freshwater Snails (Mollusca: Gastropoda) of North America*. Washington, D.C.: Government Printing Office, EPA Publications. Washington, D.C.

Thorp A.P. and A.P. Covich (eds.) 1991. *Ecology and Classification of North American Freshwater Invertebrates*. Academic Press, Inc., San Diego, Ca.

Bivalvia

Burch, J.B. 1972. *Freshwater Sphariacean clams (Mollusca: Pelecypoda) of North America*. EPA-WPCRS 18050, ELD03/72. Supt. Doc. No. 5501-0367. Washington, D.C.

Thorp A.P. and A.P. Covich (eds.) 1991. *Ecology and Classification of North American Freshwater Invertebrates*. Academic Press, Inc., San Diego, Ca

Starnes, L.B. and A.E. Bogan. 1988. *The Mussels (Mollusca: Bivalvia: Unionidae) of Tennessee*. Amer. Malacological Bull. 6:19-38.

Cummings, K.S. and Mayer, C.A. 1992. *Field Guide to Freshwater Mussels of the Midwest.* Illinois Natural History Survey Manual 5. 194 pp.

Mackie, G.L. 2007. *Biology of Freshwater Corbiculid and Sphaeriid Clams of North America.* Ohio Biological Survery Bulletin New Series. Volume Number 3. Ix + 36 p.

Bogan, P.E. and Parmalee, P.W. 1998. *The Freshwater Mussels of Tennessee.* The University of Tennessee Press. Knoxville, TN

William, J.D. et al. 2008. *Freshwater Mussels of Alabama and the Mobile Basin in Georgia, Mississippi and Tennessee.* The University of Alabama Press. Tuscaloosa, AL.

ANNELIDA

Tubificidae and Naididae

Brinkhurst, R.O. and Kathman, R.D. 1999. *Guide to the Freshwater Oligochaetes of North America.* Aquatic Resources Center. Thompsons Station, TN

Brinkhurst, R.O. 1986 *Guide to the Freshwater Aquatic Microdrile Oligochaetes of North America*. Buchanan Printers, Winnipeg, Man., Canada. 259 pp.

Other Oligochaetes & Branchiobdellida

Thorp, A.P. and A.P. Covich (eds.) 1991. *Ecology and Classification of North American Freshwater Invertebrates*. Academic Press, Inc., San Diego, Ca.

Hirudinea

Klemm, D. J. 1985. *A Guide to the Freshwater Annelida (Polychaeta, Naidid and Tubificid Oligocahaeta, and Hirudinea) of North America*. Kendall/Hunt Publ. Co., Dubuque, Ia.

CRUSTACEA

Amphipoda

Thorp, A.P. and A.P. Covich (eds.) 1991. *Ecology and Classification of North American Freshwater Invertebrates*. Academic Press, Inc., San Diego, Ca.

Bousfield, E.L. 1958. *Fresh-Water Amphipod Crustaceans of Glaciated North America.* Queens Printer, Ottawa, Canada

Smith, D.G. 2001. *Pennak's Freshwater Invertebrates of the United States.* John Wiley & Sons, Inc. New York, NY

Decapoda

Hobbs, H.H., Jr. 1972 *Crayfishes (Astacidae) of North and Middle America*. EPA-WPCRS No. 18050, ELD05/72. Supt. Doc. No. 5501-0399, Washington, D.C. 173 pp.

Isopoda

Williams, W.D. 1972. *Freshwater Isopods (Asellidae) of North America*. EPA-WPCRS No. 18050 ELD05/72. Supt. Doc. No. 5501-0390. Washington, D.C. 45 pp.

INSECTA

Collembola

Merritt, R.W., K.W. Cummins and M.B. Berg (eds.). 2008. 4th Edition. *An Introduction to the Aquatic Insects of North America*. Kendall and Hunt Publishing Co., Dubuque, Ia.

Ephemeroptera

Merritt, R.W., K.W. Cummins and M.B. Berg (eds.). 2008. 4th Edition. *An Introduction to the Aquatic Insects of North America*. Kendall and Hunt Publishing Co., Dubuque, Ia.

McCafferty W.P. 1990. *Revisionary Synopsis of the Baetidae (Ephemeroptera of North and Middle America.* Trans. Amer. Entomol. Soc. 105(2):139-221

Brigham, A.R., W.V. Brigham and A. Gnilka (eds.). 1982. *Aquatic Insects and Oligochaetes of North and South Carolina*. Midwest Aquatic Enterprises, Mahomet, Ill.

Plecoptera

Merritt, R.W., K.W. Cummins and M.B. Berg (eds.). 2008. 4th Edition. *An Introduction to the Aquatic Insects of North America*. Kendall and Hunt Publishing Co., Dubuque, Ia.

Stewart, K.W. and B.P. Stark. 1988. *Nymphs of North American Stonefly genera (Plecoptera)*. Monograph 12. Thomas Say Foundation. 460 pp.

Odonata

Merritt, R.W., K.W. Cummins and M.B. Berg (eds.). 2008. 4th Edition. *An Introduction to the Aquatic Insects of North America*. Kendall and Hunt Publishing Co., Dubuque, Ia.

Louton, J. A. 1985. *Lotic Dragonfly (Anisoptera: Odonata) Nymphs of the Southeastern United States: Identification, Distribution and Historical Biogeography*. University Microfilms International. Ann Arbor, Me.

Needham, J.G. et al. 2000. *Dragonflies of North America.* Scientific Publishers. Gainesville, FL

Westfall, Jr. M.J. and May, M.L. 1996. *Damselflies of North America.* Scientific Publishers. Gainesville, FL

Hemiptera

Merritt, R.W., K.W. Cummins and M.B. Berg (eds.). 2008. 4th Edition *An Introduction to the Aquatic Insects of North America*. Kendall and Hunt Publishing Co., Dubuque, Ia.

Brigham A.R., W.V. Brigham and A. Gnilka (eds.). 1982. *Aquatic Insects and Oligochaetes of North and South Carolina*. Midwest Aquatic Enterprises, Mahomet, Ill.

Megaloptera

Merritt, R.W., K.W. Cummins and M.B. Berg (eds.). 2008. 4th Edition. *An Introduction to the Aquatic Insects of North America*. Kendall and Hunt Publishing Co., Dubuque, Ia.

Trichoptera

Merritt, R.W., K.W. Cummins and M.B. Berg (eds.). 2008. 4th Edition. *An Introduction to the Aquatic Insects of North America*. Kendall and Hunt Publishing Co., Dubuque, Ia.

Wiggins, G. B. 1996. *Larvae of the North American Caddisfly Genera (Trichoptera)*. 2nd ed. Univ. Toronto Press, Canada.

Lepidoptera

Merritt, R.W., K.W. Cummins and M.B. Berg (eds.). 2008. 4th Edition. *An Introduction to the Aquatic Insects of North America*. Kendall and Hunt Publishing Co., Dubuque, Ia.

Brigham A.R. and D.D. Herlong. Aquatic and Semi-Aquatic Lepidoptera. pp. 12.5-12.36 in in A.R. Brigham, W.V. Brigham, and A. Gnilka (eds.). Aquatic Insects and Oligochaetes of North and South Carolina. Midwest Aquatic Enterprises, Mahomet, Ill.

Coleoptera

Merritt, R.W., K.W. Cummins and M.B. Berg (eds.). 2008. 4th Edition. An Introduction to the Aquatic Insects of North America. Kendall and Hunt Publishing Co., Dubuque, Ia.

Brigham, A.R., W.V. Brigham and A. Gnilka (eds.). 1982. *Aquatic Insects and Oligochaetes of North and South Carolina*. Midwest Aquatic Enterprises, Mahomet, Ill.

Ciegler, J.C. 2003. *Water Beetles of South Carolina.* Clemson University Public Service Publishing. Clemson, SC

Diptera

Chironomidae

Merritt, R.W., K.W. Cummins and M.B. Berg (eds.). 2008. 4th Edition. *An Introduction to the Aquatic Insects of North America*. Kendall and Hunt Publishing Co., Dubuque, Ia.

Epler, J.H. 2001. *Identification Manual for the Larval Chironomidae (Diptera) of North and South Carolina.* North Carolina Department of Environment and Natural Resources, Raleigh, NC.

Epler, J.H. 1995 (Rev.). *Identification Manual for the Larval Chironomidae (Diptera) of Florida*. Florida Dept. Env. Prot., Tallahassee.

Wiederholm, T. (ed.) 1983. *Chironomidae of the Holarctic Region. Keys and Diagnoses. Part 1: Larvae*. Suppl. 19, Entomological Scandinavica. 457 pp.

Wiederholm, T. (ed.). 1986. *Chironomidae of the Holarctic Region. Keys and Diagnoses. Part 2: Pupae*. Suppl. 28. Entomologica Sandinavica. 457 pp.

Other Diptera Families

Merritt, R.W., K.W. Cummins and M.B. Berg (eds.). 2008. 4th Edition. *An Introduction to the Aquatic Insects of North America*. Kendall and Hunt Publishing Co., Dubuque, Ia

Criteria for Taxonomic experts

(adapted from the Taxonomic Certification Program established in 2004 by the North American Benthological Society).

In order to be considered an expert an individual must meet **at least four** of the following criteria:

1. **Authorship.** Is an author/coauthor of two or more peer-reviewed publications in the group for which the applicant seeks recognition as a taxonomic/systematic expert. Or, has prepared and presented two or more papers at professional meetings focusing on the taxonomy/systematics of the group for which the applicant seeks recognition as a taxonomic expert.

2. **Academic Qualification**. Has been presented and earned a graduate degree (MS/PhD) related to the field of invertebrate taxonomy, with MS or PhD thesis focused on the taxonomy/systematics of group for which the applicant seeks recognition as a taxonomic expert. Post-doctoral employment/experience focusing on taxonomy/systematics of group for which the applicant seeks recognition as a taxonomic expert will fulfill this criterion.

3. **Employment**. Currently serves (or has previously served) in a professional capacity (e.g., at place of employment - institution, business, agency, department, company) as curator or manager of an invertebrate collection (one or more groups) including that for which the applicant seeks recognition as a taxonomic/systematic expert.

4. **Experience**. Has a history of / currently is performing taxonomic identification / verification services for individuals, businesses, agencies, companies, and/or organizations outside of primary place of employment in the group for which the applicant seeks recognition as a taxonomic/systematic expert.

5. **Teaching**. Has organized, prepared, and successfully presented one or more taxonomic training workshops focusing on the group for which expertise is sought; the workshop or course must have been inclusive of the group for which the applicant seeks recognition as a taxonomic/systematic expert. Or, has served as an individual or as a collaborative instructor in the teaching of one or more college or university courses focusing on the taxonomy of one / several group(s) of aquatic macroinvertebrates; the course must have been inclusive of the group for which the applicant seeks recognition as a taxonomic/systematic expert.

6. **Influence and Recognition**. Has served / currently is serving as a peer-reviewer for one or more manuscripts received from a journal editor prior to its publication in the primary literature, with focus of the manuscript(s) on the group for which taxonomic expertise is sought. Service as a guest or assistant editor for a journal publishing peer-reviewed articles focusing on taxonomic / systematic issues shall satisfy this criterion.

7. **Research**. Has submitted (as PI, co-PI, or collaborating researcher) one or more proposals to (currently pending at time of request for recognition as expert) or has received research funds (grant/contract/gift) from provincial, federal, state, regional, and/or private sources that support taxonomic/systematic studies in the group for which the applicant seeks recognition as a taxonomic/systematic expert.

TAXONOMIC SPECIALISTS USED FOR REFERENCE VERIFICATIONS
Note only those designated current are still available for verifications

Oligochaeta

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Sarasota, FL

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Springfield, OH

Mollusca

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N.C. State Museum of Natural Sciences
Raleigh, NC

Odonata

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256-766-6970

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Marshall University Department of Biological Science

Hemiptera

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National Museum of Natural History, Smithsonian Institute

Paul K. Lago, PhD
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Ephemeroptera

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Beltsville, MD 20705-2350
301-504-7041
asolis@sel.bare.usda.gov

Chironomidae

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461 Tiger Hammock Rd.
Crawfordville, FL 32327
johneplr@freenet.tlh.fl.us
850-926-3700

Simuliidae

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Beltsville, MD

Ceratopogonidae

William L. Grogan, PhD (Retired)
Cooperating Scientist for Systematic Entomology Lab., CTSU
Beltsville, MD

Steve Murphee, PhD
Dept. of Biology, Belmont University

Empididae and **Stratiommyidae**

Norman E. Woodley (current)
Systematic Entomology Lab, CTSU
Bldg. 046, BARC-West
10300 Baltimore Ave.
Beltsville, MD 20705-2350

Athericidae, Blephariceridae, Dixidae, Ephydridae, Psychodidae, Sciomyzidae

No specialists available.

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