Standard Operating Procedures (SOP):
Aquatic Field Collection Guidelines
Habitat Characterization
Benthic Sample Processing

Microscopy Laboratory 486
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PROLOGUE

The following document was originally structured to adhere to laboratory Quality Assurance (QA) Quality Control (QC) procedures and data audits required through a funding agreement with the Environmental Protection Agency (EPA). The Microscopy Lab, or BugLab (Room 486), at the University of Minnesota Duluth, Natural Resources Research Institute (UMD, NRRI) amended previously established benthic sample processing guidelines into a NRRI Technical Report to satisfy a grant requirement for a 1994 Ecocriteria Project (189-6074). The Standard Operating Procedure (SOP) version 1.0 document included general guidelines for sampling, transferring, processing, identifying, and summarizing data for benthic samples at NRRI's BugLab. Due to adjustment made to meet requirements on various projects, this SOP is not specific, and only provides a general overview in protocol. When necessary, detailed descriptions for individual project procedures are amended to this document under the Project Protocol section.

This document (NRRI Technical Report, NRRI/TR-99/37) is cited as;

Natural Resources Research Institute (NRRI). 1999. Standard Operating Procedures (SOP): Benthic Sample Collection and Processing, University of Minnesota Duluth, Natural Resources Research Institute, Technical Report, NRRI/TR-99/37, 17 p.

OR

Breneman, D. 1999. Standard Operating Procedures (SOP): Benthic Sample Collection and Processing, University of Minnesota Duluth, NRRI Technical Report, NRRI/TR-99/37, 17 p.

INTRODUCTION

Assessing biological condition of aquatic habitats involves multiple phases and a variety of methodologies and techniques. Monitoring, as one form of assessing biological condition includes, but is not limited to, field observations, data and sample collection, laboratory processing, and data interpretation. This document is limited to aquatic biota sampling, characterizing habitat structure, assessing aquatic macrophytes and adjacent riparian condition, and evaluating water quality parameters. Both quantitative and qualitative methods are used to collect biological samples and evaluate habitat characteristics for interpreting, or continually monitoring, biological condition. Guidelines are provided to establish sample collection protocols once an experimental design is formulated. Descriptions that follow will be limited to habitat characteristic evaluation, monitoring water quality parameters, and fish, macroinvertebrate, and periphyton community sampling.

University of Minnesota Laboratory Safety Standards

General field sampling and safety requirements are provided in this document, in addition to an overview of the sample processing procedures utilized in the Microscopy Laboratory at the UMD-NRRI. Prior to any work related effort, individuals will have attended First Aid, Field, and Boater Safety seminars, met MERTKA and laboratory safety training requirements, and been approved by NRRI's safety officer. For detailed information regarding general laboratory safety, protocols, and laboratory chemical inventory (Table 1), or refer to the University of Minnesota Department of Health and Safety, Laboratory Safety Plan (see http://www.nrri.umn.edu/safety/policies.html for documentation).

Table 1. Microscopy Laboratory chemical inventory and documentation.

Microscopy Laboratory Sample Storage And Chemical Inventory		
Chemical/Product	*CAS#	NRRI Room No.
Formaldehyde Solution (37%)	50-00-0	486, 488, 106
Ethyl Alcohol (ETOH, 190 Proof)	64-17-5	486, 488, 106
Euparal (Eucalyptol)		486
Permount Solution		486
CMC 10 Mounting Media		486
Sodium Nitrate	7631-99-4	486
Kahle's Perservative (75% Formalin, 25% ETOH v/v)		106
LUDOX (Silicoidal Solution)		106
Sodium Phosphate	7558-79-4	486
FINQUEL (MS-222)	886-86-2	486

*Material safety data sheets for any chemicals/products are in the general Laboratory Standard Operating Procedure manual (NRRI/1999-TR/37) located in NRRI Rm. 486.

METHODS

Field data collection includes a variety of parameters evaluated at each sampling location. General procedures are provided, and include adaptations of previous NRRI surveys (c.f., Richards *et al.* 1997, Breneman *et al.* 2000, Johnson *et al.* 2003, and Brady *et al.* 2004). Protocols for various

study designs (described in amended Protocols A and B), Great Lakes nearshore, coastal, protected, and riverine wetlands (Protocol C), and isolated wetland and temporary pool invertebrate sampling procedures (Protocol D).

Habitat Characteristic

A variety of physical, chemical, and ecological parameters are evaluated to summarize aquatic habitat characteristics (see Appendix G, H, and Protocol D). Wadable streams include a sample reach that should be at least 50 to 100 m from a road crossing or man-made obstruction, unless it is part of the study design. Reach length is dependent on the average stream width and should include as many riffle-run sequences as possible. To obtain an estimated stream reach length, multiply mean stream width by 15-35 to establish a total length. Transects are used to evenly space point estimates for various observations (Figure 1). Transects are established across the channel perpendicular to flow. A minimum of 10 transects placed at 10 meter intervals (110 m minimum reach length) to evaluate fish habitat, or to accommodate narrow, homogenous stream conditions. Transects are spaced evenly based on total reach length. A preliminary walk-through should take place prior to establishing the boundaries for sample collection. A schematic stream reach diagram noting habitat characteristics, and a cross-sections diagram at each transect should be completed.

Transect Points- Point estimates are used to evaluate stream feature, discharge rates, substrate type and proportional coverage, substrate embeddedness by fine particles, in stream habitat cover, bank slope and stability, riparian condition, and riparian corridor extent. Five points evenly spaced along each transects centers a 0.25 m^2 grid, and is used to quantify substrate size categories and

composition (% coverage).

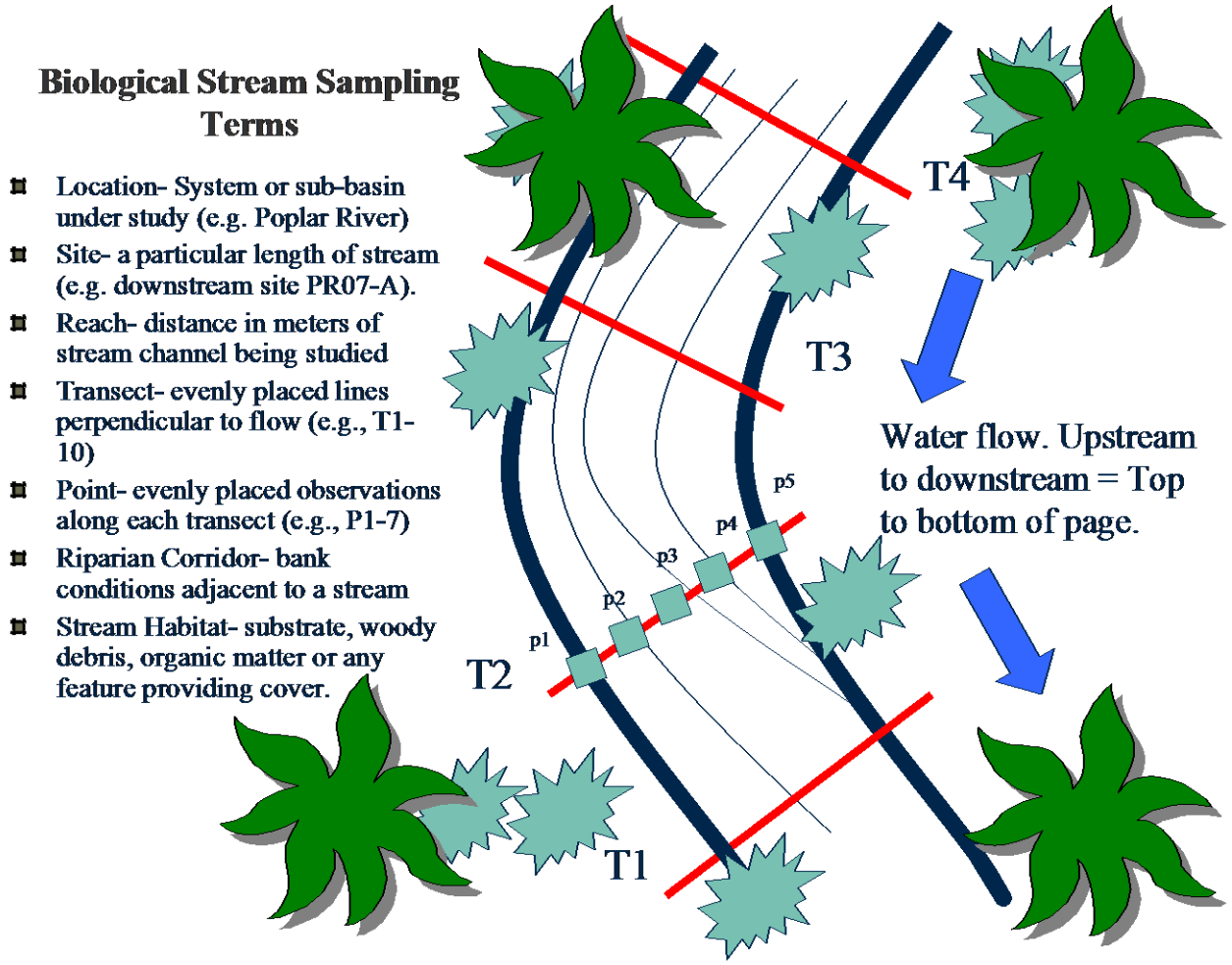

Figure 1.

Substrate- Within each grid, the extent (in percent surface area covered) and type of substrate particles are estimated. Classifications schemes should adhere to standardized particle size categories (c.g. Brusven and Prather 1974, Friedman and Sanders 1978, Gee and Bauder 1986). The extent large substrate particles are embedded by fine particles (sand and silt) is also measured (as percent embedded) at one point within each grid. An additional sediment depth measurement along each transect is recorded to determine the maximum extend of fine particle deposition using a sediment rod. This point is not random, but a subjective choice based on the

amount fine particle accumulation. This measurement can be repeated to obtain a maximum reading, but only one measurement is record per transect. A sediment sample will be collected at these locations for later particle size analysis. Approximately 300 cm^3 of sediment from each habitat zone will be composited across transects (typically 4 to 6 transects). Composite samples (approximately 1200 - 2000 cm^3 per habitat zone) will be labeled and stored on ice and/or frozen prior to analysis.

Flow- Stream discharge is estimated from flow recordings and depth collected for specific reasons. A 15-20 point cross-sectional estimate is made in an appropriate area. Those data are separate from a series of 10-second flow-weighted averaging (FWA) estimates along the random transects to evaluate fish habitat conditions. Instructions for FWA are provided in the Marsch-McBirney Flow-mate operators' manual. Water depth and flow rate are recorded at a depth 20% above the substrate floor. A top-set wading rod is used to automatically set the proper position of stream flow sheer strength.

In stream cover- When transect lines intersect instream habitat cover, note type, size, and stability on the data sheet. Schematic diagrams of the size, shape, and dimensions of habitat cover such as large boulders, islands, etc., should be recorded. Large woody debris (greater than 1 m in length and 10 cm dia.), debris dams, roots wads, etc. that intersects a transect is recorded in detail, noting length or surface area, stability, and position along the transect. Total amount of woody debris per reach is also estimated by counting the number of intact units (100 cm in length by 10

cm dia). A reach survey Qualitative Habitat Evaluation Index (Ohio EPA, 1987) to rank overall stream condition is also recommended for each site following the sampling event.

Bank structure- Bank or shoreline structure and condition (stable or unstable) is evaluated on all transects with depth of undercut bank recorded in those instances. Bank full width and substrate composition are recorded, as well as high water marks or indicators of flood extent. Erosion estimates guidelines are included as an amended protocol (Protocol E) or as second tier observations (more intensive surveys). Bank slope will determined by calculating the rise over run distance at selected points from wetted to bank full width using a magnifying level and survey staff.

Riparian corridor – Densiometer readings at a mid-stream point in four cardinal directions on each transect provides an estimate of stream shading potential. Riparian width is measured and bank vegetation type characterized for 0-10 m adjacent to the stream. Riparian type (ranked categories), percent composition, and where applicable, maturity (by recording a series of diameter at breast height measurements), provide an indication of channel stability and succession. Adjacent riparian and landuse characteristics from 10-30 m and beyond are estimated using GIS classification categories. For whole-reach summary guidelines, refer to Protocol D and Appendix H for more detailed descriptions of observations.

Water Quality Parameters

Water chemistry parameters at each location are recorded with a YSI 556 multi-probe meter to

establish baseline information on water temperature, dissolved oxygen, conductivity, pH, and ortho-phosphate during the sampling effort. Water clarity is determined by sampling in triplicate with seechi disc or turbidity tube readings. Depending on objectives, grab samples are collected and returned to the Analytical Lab for processing following standard protocols (Ameel.1998).

Periphytic Assemblage

Epilithic algal (periphyton) biomass is measured by surface area of AFDM and chlorophyll-a. Chlorophyll *a* accrual will be measured on nutrient-diffusing substrata after 3 weeks of colonization from all reaches using the method of Gibeau and Miller (1989) for consistency with biomass and biomass accrual rates measured by Wold and Hershey 1999, and Axler et al. (In prep.). Coarse particulate organic matter (CPOM) standing stocks will be measured from benthic macroinvertebrates samples. Ash-free dry mass (AFDM) of sorted material >1 mm will be determined by placing samples in a muffle furnace at 500ºC for 2 hr. Filamentous green algae will be removed before burning. Large wood standing stocks will be assessed via methods of Johnson et al. 2003.

Fish Sampling

The fish sampling effort is conducted during base flow conditions. Stream fish assemblages are sampled using DC-pulsed gear outfitted with either a tote-barge or a portable backpack unit. The type of gear used is dependent on stream depth, width, and substrate type. Electrofishing begins from downstream, moving upstream with a 3-person crew recommended. Each crewmember

should be outfitted with wading boots, rubber gloves, and polarized sunglasses. Backpack shocking can be accomplished with a 2-person crew, but 3 individuals are required for tote-barge operation. When backpack shocking with 3 crewmembers, the lead individual moves in front with the anode and dip net. The second individual then carries a dip-net and the back-pack unit following closely behind the lead. The third individual is responsible for maintaining a cooler for recovered fish and monitoring fish condition. If a 2-crewmember effort is attempted, the lead effort remains as described above, and the second individuals netting responsibilities are replaced with transporting and monitoring recovered fish. Tote-barge shocking requires 3 individuals, with 2 crewmembers moving out in front in staggered increments, returning and shocking back toward the barge every 10 m to deposit recovered fish. The third crewmember is responsible for maintaining the tote barge, recovered fish, and equipment. Anode cables connecting the lead individuals must remain unobstructed, and recovered fish can often be transferred to the barge from the leads net to a larger net and into the cooler on the tote barge. The third individual monitors recovered fish condition, generator and shocking equipment operation, and path of tote barge. The reach length included in stream surveys is generally based on 10-15x the stream width, but a minimum of 100 m is sampled. A single-pass method is adequate to establish an estimate of taxa richness within each sample reach but is specific to project protocol. Due to constraints with the electrofishing equipment, wetland habitats are sampled with 24-hour Fyke net sets. Large-framed (LG-0.9x1.2m with a 0.9 m box and 12 mm mesh) and small-framed (SM- 0.45x0.75 m with a 0.45 m box and 4 mm mesh) nets are used for passive collection. Both designs included 3 m wings and 8 m leads.

All individual fish recovered are identified to species, divided into age classes when necessary (e.g., adult, juvenile, young of the year), enumerated, measured (total length in mm), and a minimum of 25 individuals weighed (in grams) per group. It is recommended that all individuals be measured and weighed (with the portable balance scale recorded), but exact numbers are dependent on specific protocols. Individuals retained for further identification are preserved in Kahle's solution and returned to the laboratory. If a few individuals are preserved to represent a larger catch of unknowns released on site, those unknowns are placed in a separate vial. With the exception of unknown species, all individuals are measured and released a minimum of 50 m downstream if shocking effort is to continue. Following positive identification of unknowns, specimens returned to the lab are either retained in a reference collection (Rm 486, NRRI) or disposed of via incineration. Catch per unit effort (CPUE) is calculated for each sample location. Streams CPUE is determined by standardizing abundance values by reach volume, and fyke net catch is standardized by net sampling hours.

Fish identifications and traits data are referred to the following sources: (Eddy, Becker, Scott and Crossman)

Macroinvertebrate Sampling

A multi-habitat sampling approach is used to evaluate macroinvertebrate (Lenat 1988) and periphytic assemblages. Various gear types are available to sample benthic invertebrates, and each method is designed to maximize sampling effectiveness based on habitat characteristics available. Benthic samples are collected in the field following protocols established in the study design (see

appropriate Protocol Appendices), and returned to the laboratory for processing. Sample processing involves; 1) washing and sub-sampling material, 2) physically separating aquatic fauna from inorganic and other organic materials in the sample, 3) sorting or preparing organisms, and 4) identify and enumerating individuals. Sample processing procedures primarily involve 5 steps: 1) Field Sampling, 2) Sample Inventory, 3) Sample Processing 4) Sample Identification, and 5) Data Entry.

Benthic samples are collected during baseflow conditions. Quantitative samples are collected in run, riffle, and pool habitats using either a modified Hess (0.086 m^2), Surber (0.023 m^2) or Ekman grab (0.023 m^2) samplers. In wetlands, quantitative samples are collected with a petite Ponar dredge (0.023 m^2) or sediment core tube (0.0045 m^2) in shallow areas. Quantitative samples are collected at all sites, and either outfitted or washed in the field with a 254-µm mesh net or sieve. Where available, qualitative samples are collected in bank or over-hanging vegetation, woody debris dams, boulder piles or rip-rap, or from sediments and aquatic vegetation in run and pool habitats using a D-frame kick net (mesh size: 500 µm or smaller). The D-net effort should be timed and measured (approx. 30 seconds per sample and a 10 m distance). Extensive herbaceous vegetation (primarily gramenoid grasses) and instream aquatic vegetation are swept, while wood dams and boulder piles are jabbed (*sensu* Barbour et al. 1999) to dislodge invertebrates. Qualitative samples in the wetlands target the transition zone by changes in vegetation or depth. Where vegetation is available, samples are collected in the zone between the terrestrial and emergent aquatic vegetations (EAV), and between the EAV and submergent aquatic vegetation (SAV). If vegetation is absent, sample effort is stratified by depth at both 0 to 0.5 m, and from

0.05 to 1 m. The distribution of the qualitative sampling effort is to improve our ability to collect all resident taxa from as many different habitats as available. All invertebrates from each sample are collected and preserved in the field using a Kahle's preservative, 10% Formalin, or 70% ethyl alcohol (ETOH).

Sample Preservation

Samples are washed through a 250 or 500 □m mesh net or sieve and placed in a sample container, frozen, or preserved. Preservatives include; 10% formalin solution (ratio of 1 part 37% formaldehyde to 9 parts water; 1:9 v/v), 70% ETOH (ratio of 7 parts 95% ETOH to 3 parts water; 7:3 v/v), or Kahle's preservative (combination of 3 parts ETOH, 1 part 37% formaldehyde, and 4 parts water in flexible ratios of 3:1:4, v/v respectively). It is important to note that while handling this mixture protective eye wear and Nitrile gloves should be worn and procedures should take place outside, under a fume hood or in another well-ventilated area. Also, wash hand thoroughly after handling this solution.

One important aspect regarding sample collecting is to ensure that all samples are properly labeled. Each sample contains an interior and exterior label (Appendix A.). Sample labels identify: 1) project name 2) site name or number, 3) sample number and number of containers (i.e. large samples that are placed in multiple containers, A-D), 4) gear type (Hess, Ekman, etc.), 5) sieve size used to wash sample, and, 6) current date. Labels may be coded or include limited information, but must be referenced with an accompanying log sheet that provides the required information from number 1 through 6 listed above. Interior labels written in pencil lead or laser printed on non-soluble paper are required. Outside labels may also contain a brief description or code, but must include at least a unique identifier for lab sorting, prioritized processing, and archiving purposes.

Sample Inventory

A list of samples, including all label information, is completed in field notebooks as samples are collected. A sample list accompanies all samples returning to the laboratory (Appendix B-1). Chain of custody forms (Appendix B-2) are completed and verified with the field sample list as the provider and NRRI laboratory personnel inventory in-coming samples. Chain of custody forms and a field sample list are duplicated, with copies to the outside agency (where applicable), a copy filed with field notes or data sheets, and one copy placed in the project log book. Sample bottles are to be clearly labeled, logged in, approved by signature, and stored in the Crayfish Lab (Rm 126, NRRI). Archived samples and remaining sample preservatives are to be deposited in Chemical Storage (Rm. 106, NRRI).

LABORATORY PROCEDURES

Invertebrate Sample Processing

Prior to processing, samples preserved in the field with 10% formalin or Kahle's are rinsed to remove the formalin preservative. This procedure is conducted under a ventilation hood. The rinsed sample is then re-preserved in 70% ETOH. Discarded preservative are stored in containers labeled with appropriate hazardous waste information and transferred to Hazardous Chemical Storage (Rm 138a, NRRI).

Samples ready for processing are signed out of the project logbook by lab personnel (Appendix B-2). Samples may contain multiple containers, so all containers for that sample are concurrently processed. All sample information contained inside the sample container should be verified with outside labels and project log book information. Due to the amount of material contained in a sample, it may be necessary to sub-sample or fraction various samples (see Sub-Sampling below).

Samples stored in preservative (ETOH) should be removed from the sample prior to processing. ETOH is drained from the sample through the appropriate sieve and collected in a USED ETOH container under the fume hood with secondary containment, and once full is transferred to the chemical waste storage room and labeled as "Hazardous Waste". Frozen samples are usually processed for both macroinvertebrate abundance and organic biomass estimates. NO ORGANIC MATERIAL FROM THESE SAMPLES ARE DISCARDED. Processing procedures for frozen samples are project dependent and biomass estimates follow standard methods (APHA 1985, ATSM 1985).

Sample materials are washed and sorted according to specific processing procedures (see Lab Protocols attached). Large amounts of material passing through the smallest sieve are deposited in a waste receptacle and NOT discarded in the lab sink. Depending on sediment type, samples can be washed using an elutriation device, colloidal silica bath, or other floatation procedures. These devices are designed to separate light organic materials from heavier particles in a sample.

Once the sample has been thoroughly washed and sub-sampled accordingly, the sample is transferred to a tray, glass pan, or sorting dish. Sample material should be spread evenly throughout the pan. Large trays and glass pans should be sorted using a 2X magnification lens. A dissecting scope is used to process samples placed in a sorting dish (check additional protocols for specific methods).

Organisms are removed from detritus with a forceps and placed in labeled vials. Vial labels contain identical information as sample labels, with the addition of the amount of sample processed (ie, 1/4, 1/2, or whole), a vial number, the total number of vials for that particular sample (e.g., 1 of 3), and initials of lab personnel (Appendix A). The number of vials accompanying each sample will depend on the abundance of organisms, but one vial should be designated for only midge larvae (Diptera: Chironomidae).

Samples that have been thoroughly processed are subject to quality assurance/ quality control (QA/QC) guidelines. Each completed sample and accompanying shelf vials are designated as such

by placing an additional label on the container indicating its status (ie, “picked”) and date completed. All samples will be subject to QA/QC inspection (100% inspection). Rejected samples are re-processed until they pass QA/QC guidelines. Samples that pass QA/QC inspection may be discarded unless the sample is to be used to obtain organic content estimates. Those samples should be handled according to project protocol or standardized procedures (APHA 1985, ATSM 1985). Vials containing sorted organisms preserved in 70% ETOH are logged into a vial chain of custody form (Appendix C).

Sub-Samples

Samples are split when the amount of material is too large and will not allow the entire sample to be processed within a predetermined time period. Generally, sample processing, from start to finish, is completed in 3-4 hours. The 3-hour time frame for actual picking time should be considered. A quarter sample that requires less than 1 hour will result in an addition quarter, and potentially a whole sample being processed (e.g., 4 hours total). If a quarter sample requires less than 2 hours to complete, another quarter sample is completed, resulting in a minimum of 1/2 sample being processed. Only very difficult samples should remain a quarter picked. It is recommended that the process be completed in a continuous 3-4 hours time period, or by the end of a work session to avoid errors (eg. lost labels, spilled sample, ect.). No samples will remain outside of the appropriate sample jar when the responsible technician has left the laboratory.

Sub-sampling requires using a sample splitter, sectioned tray, sieve template, or partitioned basin. Sub-sampling device will depend on sample volume and content. Sub-sampled portions not being

processes are returned to the appropriate container and labeled with appropriate information in addition to the fraction remaining. Depending on the sample material and ability to homogenize the sample in the splitter, it is recommended that the ¼ fraction to be processed is a random accumulation of 2, 1/8 fractions.

Sample Indentification

Sample vials containing processed macroinvertebrates are then signed out for identification using the vial chain of custody form (Appendix C). Organisms are identified to the lowest taxonomic level using appropriate keys (Hillsenhoff, W.L. 1981, Wiederholm, T. 1983, Brinkhurst, R.O. 1986, Thorp, J.H. and A.P. Covich 1991, Merritt, R.W. and K.W. Cummins 1996), enumerated, and recorded on a identification data sheet (Appendix D). A reference collection is made for each individual taxa identified for the each project. The collection is then subject to QA/QC guidelines that include re-identifying 30% of the collection. If more than 5% of the randomly selected taxa are questioned, the entire collection is re-identified. QA/QC guidelines for re-enumeration of samples will be project dependant.

Individual Mounts

Individuals within the family Chironomidae (Diptera) are permanently mounted for further taxonomic identification (Appendix E). Generic identification requires head capsule mounts to ensure ventral viewing of individual mouth parts. The number of Chironomidae mounted is project dependent (see Project Protocols). Generally, 60-100 midge larvae are size classed into three categories and randomly selected in equal number from the total pool of individuals.

Similar sized individuals are placed on the same slide. Generally, the number of individuals per slide depends on body size. It is possible that 2 individuals can be place under the same cover slip. Individual placement on the slide and label information will follow standard template (Appendix F).

Depending on specimen condition, head capsules will either be rolled ventral side up next to the body, or the entire body cleared with clearing agent (#6373 BioQuip Products, Inc.) or CMC (Polysciences, Inc., Warrington, PA) and flattened under the cover slip. Organisms are soaked in 95% ETOH, preserved in a mounting medium such as euparal (BioQuip Products, Inc., Gardena, CA) or CMC, and placed under a cover slip.

Individual Mount Identification

Permanent slide mounts will be identified to the lowest taxonomic level under a compound microscope. As indicated by Appendix F, each individual will be assigned a particular slide number, cover slip letter, and cover position. A separate reference collection for these organisms is not necessary because location of individual organisms can be easily located between slides and data sheets. Chironomidae will also be subject to a 30% random re-identification for lab verification. QA/QC guidelines will be project dependant.

Sediment Processing

Sediment AFDW

Thawed sediment sample will be transferred to a basin and homogenized for 1 minute. A small

amount of water may be added to each sample to facilitate thorough mixing. Homogenized sediment in the mixing container will be tamped to settle material uniformly. Sediment will be sub-sampled in triplicate by extracting 250 cm^3 using a 5 cm (dia.) sediment core. Sub-samples will be placed in labeled pans and dried (105^o C) to a constant weight determined with a standard balance. Dried samples will be ignited for 1 h at 500^o C. After samples cool, reagent-grade water is added to re-wet ash and compensate for water weight not driven off from clay particles during the drying period (APHA, 1992). Samples are dried to a constant weight at 105^o C and re-weighed to determine AFDW of each sub-sample.

Substrate particle size analysis

Dried sub-samples will be run through a set of six sieves (4, 2, 0.5, 0.25, and 0.0625 mm) for 1 minute using a row-tapper to obtain six particle size fractions: 1) > 4 mm, 2) 4 - 2 mm, 3) 2-0.5 mm, 4) 0.5 - 0.25 mm, 5) 0.25 - 0.0625 mm, and 6) < 0.0625 mm (Gordon *et al.* 1992). Sediment retained in each size fraction will be transferred to weigh-boats, weighed using a standard balance, and recorded.

Data Entry

Data from laboratory sheets will be entered by Microsoft Access, a server database, or entered in duplicate using standard spreadsheet software. The data are subject to a 10% random evaluation according to the number of data records. An error rate greater than 1% will result in re-entry.

Prior to post-processing data, raw files are subjected to an invertebrate database (ie. trait information) to verify record continuity. Database invertebrate information (ie. output file referred to as “bugspec”) is queried from a server to obtain the most recent version. Those data in a spreadsheet format are then merged with the raw data via a SAS name verification program (e.g., IDdatalist.sas) to check for errors and provide higher taxonomic categories for all individuals identified. Individuals not listed in the database are either re-identified or the current information and taxonomic name are compared to the ITIS system database for confirmation (see http://www.itis.usda.gov). Once the taxa name field properly merges with the name list program, the raw data is run through a SAS file (bugspec*.sas) to generate taxa counts and abundance values for selected metric categories (taxonomic, structural, and functional traits).

LITERATURE CITED

Ameel, J. Ruzycki, E., Owen, C.J. and R. Axler,1998 (revised 2003). Analytical chemistry and quality assurance procedures for natural water, wastewater, and sediment samples Natural Resources Research Institute, University of Minnesota Duluth, MN. Technical Report NRRI/TR–98/28.

American Public Health Association (APHA), American Water Works Association, and Water Pollution Control Federation. 1985. Standard Methods for the Examination of Water and Waste Water. 16th ed. Washington, D.C. 1067 p.

American Standards for Testing and Materials (ASTM), 1985. Annual Book of ASTM Standards. Part 31; Water. Philidelphia, PA.

Barbour, M.T., Gerritsen. J., Snyder, B.D., and Stribling, J.B. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates, and fish, Second edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.

Becker, G.C., 1983. Fishes of Wisconsin. The University of Wisconsin Press, London, England.

Brady, V., L. Johnson, and J. Reed. 2004. Manitou Stream Monitoring Project: Field Methods. Report to Lake County Forestry Department and The Nature Conservancy. Natural Resources Research Institute Report NRRI/TR-2004/36.

Breneman, Dan, Carl Richards, and Steve Lozano. 2000. *Environmental influences on benthic community structure in a Great Lakes embayment. J. Great Lakes Res.* 26(3):287-304.

Brinkhurst, R.O. 1986. Guide to the Freshwater Aquatic Microdrile Oligochaetes of North America. Canadian Special Publication of Fisheries and Aquatic Sciences 84. 259 pp.

Brusven, M.A. and K.V. Prather, 1974. Influence of stream sediments on distribution of macrobenthos. J. Entomol. Soc. Brit. Columbia. 71:25-32.

Friedman, G.M. and J.E. Sanders. 1978. Principles of Sedimentology. John Wiley and Sons, New York.

Gibeau, G.G., and Miller, M.C. 1989. A micro-bioassay for epilithon using nutrient-diffusing artificial substrata. J. Freshw. Ecol. **5**: 171-176.

Gee, G.W. and J.W. Bauder, 1986. Particle-size analysis. In; *Methods of Soil Analysis, Part I. Physical and Mineralogical Methods*, A. Klute, ed. American Society of Agronomy-Soil Science Society of America, Madison, WI. Pp. 383-411.

Gordon, N.D., T.A. McMahon, and B.L. Finlayson. 1992. *Stream hydrology: an introduction for ecologists*. John Wiley and Sons, Inc. NY, NY. 526 p.

Hillsenhoff, W.L. 1981. Aquatic Insects of Wisconsin. Keys to Wisconsin genera and notes on biology, distribution, and species. Publication of the Natural History Council, University of Wisconsin-Madison, 60 pp.

Johnson, L.B., D. Breneman, C. Richards. 2003. Macroinvertebrate community structure and function associated with large wood in low gradient agricultural streams. River Research and Applications 19(3):199-218.

Lenat, D.R. 1988. Water quality assessment of streams using a qualitative collection method for benthic macroinvertebrates, J North Am. Benthol. Soc. 7:222-233.

Merritt, R.W. and K.W. Cummins, ed. 1996. An Introduction to the Aquatic Insects of North America, 3rd ed. Kendall/Hunt Publishing Co., Dubuque.

Richards, Carl, R.J. Haro, L.B. Johnson, and G.E. Host. Catchment and reach-scale properties as indicators of macroinvertebrate species traits. Freshwater Biology, 37:219-230.

Thorp, J.H. and A.P. Covich (ed.). 1991. Ecology and Classification of North American Freshwater Invertebrates. Academic Press, Inc. 911 pp.

Wiederholm, T. (ed.). 1983. *Chironomidae of the Holarctic Region*. Ent. Scand. Suppl.

Wold. A.P. and A.E. Hershey. 1999. Spatial and temporal variability of nutrient limitaion in 6 North Shore tributaries to Lake Superior. J.N. Amer. Benthol. Soc. 18(1)2-14.

APPENDIXES