
Appendix 1: NAMC Sample Processing: Subsampling, Sorting and Identification of Macroinvertebrate Samples

Document produced by Scott Miller and Sarah Judson

Last Updated: October, 2011

Table of Contents

<u>SORTING</u>	<u>2</u>
<u>TAXONOMY</u>	<u>2</u>
<u>DATA ENTRY</u>	<u>3</u>
DATA OWNERSHIP POLICY	3
<u>SAMPLE ARCHIVING</u>	<u>3</u>
<u>REFERENCES CITED</u>	<u>4</u>
STANDARD LABORATORY SORTING PROCEDURES	4
MAJOR TAXONOMIC IDENTIFICATION RESOURCES USED.....	4

Sorting

General procedures for processing invertebrate samples are similar to those recommended by the United States Geological Survey (T. F. Cuffney et al. 1993; Moulton et al. 2000) and are described in greater detail and rationalized in Vinson and Hawkins (1996). A detailed step-by-step, pictorial guide of our laboratory processing is available on our website.

Samples are sub-sampled if the sample appears to contain more than 600 organisms. Sub-samples are obtained by pouring the sample into an appropriate diameter 500 micron sieve, floating this material by placing the sieve within an enamel pan partially filled with water and leveling the material within the sieve. The sieve is then removed from the water pan and the material within the sieve is divided into two equal parts. One half of the sieve is then randomly selected to be processed and the other half set aside. The sieve is then placed back in the enamel pan and the material in the sieve again leveled and split in half. This process is repeated until approximately 600 organisms remain in one-half of the sieve.

The sub-sampled material is placed into a gridded Petri dish and examined systematically under a dissecting microscope with at least 7x magnification until all organisms are removed. As organisms within a sub-sample are removed, they are enumerated and placed into separate vials according to taxonomic Orders. Additional sub-samples are taken until at least 600 total organisms are removed.

When sub-sample sorting is completed, the entire sample is spread throughout a large white enamel pan and searched for 10 minutes to remove any taxa that might not have been picked up during the initial sample sorting process. The objective of this "big/rare" search is to provide a more complete taxa list by finding rarer taxa that may have been excluded during the sub-sampling process. These rarer organisms are placed into a separate vial and the data entered separately from the individuals removed during the sub-sampling process.

Sorted (i.e., clean) and unsorted material are retained in separate jars. The sorted material is subject to random selection for assessing sorting efficiency (Appendix 2). The unsorted material is retained temporarily to resolve any discrepancies in large subsamples (>1000 organisms), unusual lab splits, etc. Once the data had been entered into a computer and checked, the sorted and unsorted portion of the sample are discarded.

Taxonomy

All organisms removed during the sorting process are identified by Society for Freshwater Science (previously the North American Benthological Society) certified taxonomists. We strive to identify organisms to a consistent taxonomic level. Generally, we follow the Southwest Association of Freshwater Invertebrate Taxonomists (SAFIT) Standard Taxonomic Effort Level 1 guidelines in which insects are identified to genus and most non-insects are identified at coarser levels. A complete listing of our Standard Taxonomic Effort can be found on our website. Small (early instar) and poorly preserved specimens may be identified to a higher level than specified. A notable difference between SAFIT recommendations and our identification protocols is that NAMC identifies Chironomidae to subfamily. All specimens are identified to the lowest taxonomic level feasible without slide-mounting. This specification precludes our

ability to identify Chironomidae finer than subfamily, but we are well networked with other labs and regularly subcontract the Chironomidae identifications if requested.

Data Entry

Currently, all sorters and taxonomists enter their processing and identification data directly into an electronic interface which updates a central SQL database. Historically (pre-2011), sample information and invertebrate data were entered into the SQL database via a single data entry employee from taxonomic bench sheets.

Before generating reports, the data is routinely checked for missing information, outliers, and other discrepancies. Once the data is approved, it is inserted into our standard report and is made available online or via specific requests.

Data Ownership Policy

We believe that we should work cooperatively to enhance biomonitoring procedures and to maintain the quality of our nation's waters. We believe this means we should openly share ideas and data. Our policy on data ownership is that we will make all invertebrate, site, and sample data available through online query tools (<http://www.cnr.usu.edu/wmc/htm/data>) and to external requests. Unless specified otherwise in writing that the data generated from field or laboratory work is proprietary, we will make these data available to requests as soon as we make it available to the original customer following data entry and quality checks. Our policy is the same for the organisms we identify. We archive identified organisms, and unless specified in writing, we will lend these organisms to anyone who requests them, including our internal reference collection and classroom uses.

Sample Archiving

Once the data has been checked, we discard the unsorted portion of the sample unless the customer requests otherwise. The identified portion of the sample is archived in our permanent collection and kept for the foreseeable future, but if we run out of space, a five year storage limit may be imposed. Archived samples are stored in 70% ethanol in glass screw-top vials with polypropylene lids and polypropylene liners. Sample labels are written with fade proof permanent black carbon ink on waterproof paper. Information on each label includes the sampling location, sampling date and a sample ID (unique catalog number). This catalog number is maintained in a computer database. Additional information stored in this computer database includes more specificity on the locality and methods of collection, the agency and personnel who made the collection, the person who identified the specimens, and any additional information associated with a sample. Samples are stored sequentially in a metal cabinet. Retrieval of individual samples or a group of samples with select taxa or by geographic area is made possible by the integration of sample archiving and the computer database. Alternatively, if you would rather keep your samples yourself we will return them in containers as described above.

References Cited

Standard Laboratory Sorting Procedures

- Cuffney, T.F. et al., 1993. *Methods for collecting benthic invertebrate samples as part of the National Water-Quality Assessment Program*, US Geological Survey. Available at: <http://water.usgs.gov/nawqa/protocols/OFR-93-406/>.
- Moulton, S. et al., 2000. *Methods of analysis by the US Geological Survey National Water Quality Laboratory-processing, taxonomy, and quality control of benthic macroinvertebrate samples*, US Geological Survey. Available at: <http://nwql.usgs.gov/OFR-00-212.shtml>.
- Vinson, M.R. & Hawkins, C.P., 1996. Effects of sampling area and subsampling procedure on comparisons of taxa richness among streams. *Journal of the North American Benthological Society*, 15(3), p.392–399. Available at: <http://www.jstor.org/stable/1467286>.

Major taxonomic identification resources used

- Adler, P., Currie, D. & Wood, D., 2004. *The Black Flies (Simuliidae) of North America*, Cornell University Press.
- Baumann, R.W., Gaufin, A.R. & Surdick, R.F., 1977. *The stoneflies (Plecoptera) of the Rocky Mountains*, American Entomological Society.
- Brown, H.P., 1972. *Aquatic dryopoid beetles (Coleoptera) of the United States*, U. S. Environmental Protection Agency, Project # 18050, Contract # 14 12 894. Available at: <http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=9100UM19.txt>.
- Burch, J.B., 1973a. *Biota of Freshwater Ecosystems Identification Manual No. 11: Freshwater Unionacean Clams (Mollusa: Pelecypodae) of North America*, U. S. Environmental Protection Agency, Project # 18050, Contract # 14 12 894. Available at: <http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=20017Q7N.txt>.
- Burch, J.B., 1973b. *Freshwater unionacean clams (Mollusca: Pelecypoda) of North America*, U. S. Environmental Protection Agency, EPA 600\3 82 023. Contract # 68 03 1290.
- Burch, J.B., 1982. *North American Freshwater Snails: Identification keys, generic synonymy, supplemental notes, glossary, reference, index*, Environmental Monitoring and Support Laboratory, Office of Research and Development, US Environmental Protection Agency. Available at: http://molluskconservation.org/WALKERANA/walkerana_vol1_no4_1982.PDF.

- Burch, J.B., 1988. North American freshwater snails: introduction, systematics, nomenclature, identification, morphology, habitats, distribution. *Walkerana*, 2, p.1-80.
- Burch, J.B., 1980. North American freshwater snails: species list, ranges, and illustrations. *Walkerana*, 1(3), p.1-215. Available at:
http://molluskconservation.org/WALKERANA/walkerana_vol1_no3_1980.PDF.
- Dillon, R. ed., 2006. *Pacific Northwest Taxonomic Workshop - Gastropoda*, Missoula, MT: The Xerces Society.
- Gelhaus, J.K., 2008. *Manual for the Identification of Aquatic Crane Fly Larvae*, Salt Lake City, UT: North American Benthological Society Taxonomic Workshop.
- Gelhaus, J.K., 2000. *Manual for the Identification of Aquatic Crane Fly Larvae for Northwest North America*, Ellensburg, Washington: The Northwest Biological Assessment Workgroup's Taxonomic Workshop on Diptera. Available at:
http://clade.ansp.org/entomology/people/gelhaus/nw_crane-fly_manual.pdf.
- Jacobus, L.M. & Randolph, P., 2005. *Mayflies in Moscow: Northwest Ephemeroptera Nymphs*, Northwest Biological Assessment Workshop, 9th Annual Taxonomic Workshop.
- Klemm, D.J., 1985. *A guide to the freshwater Annelida (Polychaeta, naidid and tubificid Oligochaeta, and Hirudinea) of North America*, Dubuque, Iowa: Kendall Hunt Publishing Co.
- Klemm, D.J., 1972. *Biota of Freshwater Ecosystems Identification Manual No. 8, Freshwater Leeches (Annelida: Hirundinea) of North America.*, Available at:
http://www.epa.gov/bioiweb1/pdf/EPA-905-R-72-117FreshwaterLeeches_Klemm.pdf.
- Larson, D., Alarie, Y. & Roughley, R., 2000. *Predaceous diving beetles (Coleoptera: Dytiscidae) of the Nearctic Region, with emphasis on the fauna of Canada and Alaska*, Ottawa: NRC CNRC Press.
- Menke, A., 1979. The Semiaquatic and Aquatic Hemiptera of California (Heteroptera: Hemiptera). *Bulletin of the California Insect Survey*, 21, p.16-24.
- Merritt, R.W., Cummins, K.W. & Berg, M.B., 2008. *An Introduction to the Aquatic Insects of North America* 4th ed., Kendall Hunt Publishing. Available at:
<http://www.amazon.com/dp/075756321X>.
- Needham, J.G., Westfall, M. & May, M.L., 2000. *Dragonflies of North America*, Gainesville, Florida: Scientific Publishers, Inc.
- Pennak, R.W., 1989. *Freshwater Invertebrates of the United States* 3rd ed., New York: John Wiley and Sons, Inc.

- Perez, K.E., Clark, S.A. & C, L. eds., 2004. *Freshwater Gastropod Identification Workshop: A Primer to Freshwater Gastropod Identification*, University of Alabama, Tuscaloosa, AL: Freshwater Mollusk Conservation Society.
- Post, D., 2005. *Dytiscidae of California*, California State University - Chico, Chico, CA: CAMLnet Taxonomy Workshop.
- Rogers, C.D., 2005. *Identification Manual to the Freshwater Crustacea of the Western United States and Adjacent Areas Encountered During Bioassessment*, EcoAnalysts, Inc. Available at: <http://ecoanalysts.myshopify.com/products/crustacea-manual>.
- Smith, S.D., 1998. *Key to western Rhyacophila larvae*, Central Washington University, Ellensburg, WA: Northwest Bioassessment Working Group Taxonomic Workshop.
- Stewart, K.W. & Stark, B.P., 2002. *Nymphs of North American stonefly genera (Plecoptera)* 2nd ed., Denton Texas: University of North Texas Press.
- Thorp, J.H. & Covich, A.P., 2009. *Ecology and Classification of North American Freshwater Invertebrates* 3rd ed., Academic Pr. Available at: <http://www.amazon.com/dp/0123748550>.
- Westfall, M.J. & May, M.L., 1996. *Damselflies of North America*, Gainesville, Florida: Scientific Publishers, Inc.
- Wiggins, G., 1996. *Larvae of the North American caddisfly genera (Trichoptera)* 2nd ed., Available at: <http://www.amazon.com/dp/0802027237>.