NAMC Sample Processing: Pictorial guide to Subsampling and Sorting of Macroinvertebrate Samples

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Introduction

This document contains detailed explanation of our subsampling and sorting procedures, including picture documentation. The general procedures we follow for processing samples are similar to those described by the USGS NAWQA program (T. F. Cuffney et al. 1993; Moulton et al. 2000). Our procedures are also described in Vinson and Hawkins (1996).

Subsampling

1. Select an appropriately-sized, clean 500-micron sieve:

2. Pour the preservative from the sample through the sieve into the laboratory waste container:

3. If the sample contains a lot of sand and gravel you will need to separate the organic matter from these inorganic particles (i.e., elutriation). To do this, pour the entire sample from the sieve into a bucket. Partially fill the bucket with water. Swirl the bucket so that the organisms and organic matter become suspended in the water column and the heavier sand and gravel fall to the bottom.

Carefully decant the water and floating organisms back through a 500-micron sieve.

Continue to add water to the bucket and swirl and decant until **NO ORGANIC MATTER** remains in the bucket. When finished, closely examine the remaining material in the bucket and pick out any caddisflies, snails, clams, or other organisms that remain. Add these organisms to those on your sieve. Indicate on the sample tracking computer interface if elutriation is performed. **Note that this procedure is not a substitute for examining all organic matter at 7x using a dissecting microscope. Elutriation should not be done to separate organisms from organic matter.**

4. Keep the sample in the sieve and rinse the sample under the faucet to wash additional fine particles and silt into the sink.

5. Place the sieve in an enamel pan or bucket that is partially filled with water and "float" the sample so that it becomes level within the sieve (i.e., all organic and inorganic material is homogenously spread throughout the sieve).

Once leveled, carefully remove the sieve from the enamel pan.

6. Place an appropriately sized separator bar into the sieve to split the material in the sieve in half.

Make sure it appears that there is an equal amount of sample material in each half. Sticks and other materials should be cut along the center line prior to splitting the sample if needed.

7. Flip a coin to determine which half of the sample is to be processed; heads = right or top, tails = left or bottom.

Keep the portion of the sample to be processed in the sieve. Place the other half into a cup using a spoon and/or rinse the material into the cup using an alcohol filled squeeze bottle, ensuring all material has been removed from the sieve and separating bar Cover the cup with ParaFilm and write the portion or split of the sample on the lid, e.g., 50%.

8. If you judge that you will start with less than 50% of the sample, place the sieve back in the enamel pan and re-float the material to level it. Re-flip the coin and divide this portion in half again. Place the material you are not going to immediately sort through in a different cup, cover with ParaFilm, and label with the split percentage, e.g., 25%. Repeat this process until it appears that approximately 600 organisms remain in one-half of the sieve.

It is best to start with small splits to assess how many bugs you will find (3.125%, 6.25%). The goal is to sort at least 600 organisms from the sample. **Once you start a split you must finish it in its entirety.** If you start to sort through a split and realize that this split will contain considerably more than 600 organisms, e.g., 800-1000 or more critters; stop and pour all the material, including the bugs you have already removed from the sample back into a sieve and split it down to a more appropriate percentage.

Sorting

1. To remove organisms, all material is examined under a dissecting microscope at 7x magnification. The material under the microscope is illuminated by a fiber optic light source.

A complete sorting station is setup as follows:

2. The material to be sorted is placed little-by-little (i.e., there should never be greater than two vertical layers of material [e.g., gravel, leaves] in the petri dish at one time) into a petri dish and all organisms within the petri dish are removed Petri dishes are divided into sections by parallel lines drawn (maximum of 1.5 cm spacing) on the bottom of the dish with a permanent marker.

All sorters **MUST** use petri dishes with grids! The lines subdivide the dish so that you can systematically move across the dish. Move along these "guides" removing all organisms that you encounter.

It is sometimes helpful to swirl (i.e., elutriate) the material to be sorted prior to placing it into the petri dish. This will separate many of the organisms from the organic and inorganic material and make it easier to process the sample. Note that all organic and inorganic material still needs to be thoroughly examined at 7x magnification.

3. You need to remove **ALL** organisms from the debris, but not all organisms will be counted. Remove, but do not count the following in your 600 minimum count:
    a. damaged and immature organisms
    b. adult insects [excluding water-dwelling adults such as beetles (Coleoptera) and true bugs (Hemiptera)]
    c. zooplankton
    d. Collembola
    e. pupae
    f. worms
    g. terrestrial insects
    h. fish

    The following items do not need to be counted or removed from samples:
    a. Molt skins (i.e., exuviae)
    b. Eggs
    c. Empty snail shells

4. As you remove the organisms count them using a clicker

and drop them into vials separated by the major taxonomic orders, i.e., Diptera, Coleoptera, Ephemeroptera, Plecoptera, Trichoptera, other insects, and non-insects.

5. Identify Chironomidae (Diptera) to subfamily. All sorters must be trained by the head sorter or a taxonomist before doing Chironomidae identifications. Use head capsule characteristics to identify the three major subfamilies. Chironominae are identified by two vertically spaced eyespots and a toothed mentum. Orthocladinae are identified by two horizontally spaced eyespots and a toothed mentum. Tanypodinae are identified by a single comma-shaped eyespot, a ligula, and long retractile antennae. Place any organisms which cannot be clearly identified to subfamily in the Diptera vial for taxonomists to examine further. Resources for learning Chironomidae subfamily identification include:
    - The Buglab reference collection
    - Identification manuals (such as Merritt et al. 2008)
    - Internet resources:
        - http://www.waterbugkey.vcsu.edu/php/genuslist.php?idnum=7&o=Diptera&f=Chironomidae
        - http://h2o.enr.state.nc.us/esb/BAUwww/chiron_manual/intro.pdf
        - http://www.epa.ohio.gov/portals/35/documents/MidgeLarvaeKeyJune2007.pdf

6. Additional portions of the sample (splits) are sorted until at least 600 organisms are found. Once you start a split, remove all the organisms within it. After you think all organisms have been removed from a portion of the sample, swirl the petri dish contents and perform a final check. Repeat this process until ALL organisms are removed. It is ok if you exceed 600 organisms, but you should not exceed 1000 organisms. If your final count exceeds 800-1000 organisms, pour all the material, including the bugs you have already removed from the sample back into a sieve and split it down to a more appropriate percentage.

7. When you have removed a minimum of 600 bugs, perform a "Big/Rare" search. This search is called a "Big/Rare" search because we tend to find larger individuals, but the goal is to collect rarer taxa that may not have been present in the split samples. Spread the entire un-sorted portion of the sample into an appropriately sized white enamel pan. Place this pan under the lighted magnifier lamp. Systematically search the pan and remove any organisms that you did not find in your split samples. Perform this search for 10 minutes.

If you are in doubt that a critter is new, it is much better to pick up duplicates than to miss a bug. Put these bugs into a separate vial labeled "B/R" for "Big/Rare".

8. Print a sheet of labels onto waterproof paper. If you would like a copy of this form, email buglab@usu.edu for a copy of the form, or mimic the following format. The label should contain the following information:

    a. Sample #

    b. Sample set/Customer name

    c. Station ID or full site name

        i. In the Buglab, use the StationID written on the standard sticker placed on top of the original bottle when the sample is received in the lab.

    d. County, State

    e. Date Collected

    f. Sorter, Date Sorted, Total split percent, Total # organisms sorted

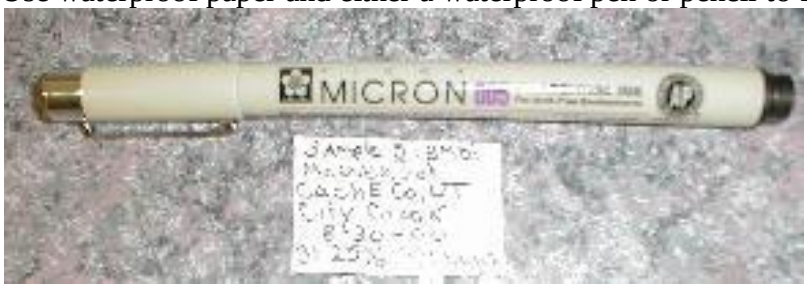
    g. Identifier, Date Identified

    h. Counts of Chironominae, Orthocladiinae, Tanypodinae

Example:

Sample #3
Wasatch-Cache National Forest
Wood Camp- Logan River,
Cache County, UT
Collected 12/12/03
BB, 12/23/03, 25%, 616 bugs
MT, 01/05/04
5 C, 12 O, 20 T

9. Use waterproof paper and either a waterproof pen or pencil to fill in the label.



10. Put the label into the Chironomidae vial and mark lid with a permanent marker.

11. Place a smaller label with the sample number (#3, from the example above) in each vial and label the lids of the vials with this same number using a dry erase marker. Label the "Big /Rare" vial "BR".

12. Put a rubber band around the vials and put them on the appropriate shelf for that customer or project. This makes the sample available for taxonomists to identify.

13. All samples are eligible to have QA/QC performed to check for sorter efficiency in removing organisms. Keep sorted material separate and label it including the word "sorted". Also keep the unsorted material in a separate jar and label it "unsorted". The material will be discarded only after the organisms have been identified, the sample data is approved, and the data has been sent to the customer. Place the sorted and unsorted jars on the appropriate shelf for that customer or project.

14. Record your sample information, including sort time, the sort dates, and your initials in your personal lab notebook and in the sample tracking computer interface. Carefully match your Sample #, BoxID (related to that customer or project), StationID, and Sample Date (date collected) to the available samples in the computer interface dropdown. The BoxID is written on the accompanying folder and shelf location for the sample set. The Sample # and StationID are on a sticker on top of the original sampling bottle. If you encounter any data entry problems, contact the taxonomists or database administrator.

15. All the organisms removed during the sorting process are then identified by qualified taxonomists. We strive to identify organisms to a consistent taxonomic level. Small (early instar) and poorly preserved specimens may be identified to a higher level than specified. Insects are normally identified to genus, some to species, and others to family. Non-insects are identified to the lowest taxonomic level feasible without slide-mounting.

References

Cuffney, T.F. et al., 1993. *Methods for collecting benthic invertebrate samples as part of the National Water-Quality Assessment Program*, US Geological Survey, http://water.usgs.gov/nawqa/protocols/OFR-93-406/.

Merritt, R.W., Cummins, K.W. & Berg, M.B., 2008. *An Introduction to the Aquatic Insects of North America* 4th ed., Kendall Hunt Publishing.

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, 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.