

Washington State Department of Ecology

Environmental Assessment Program

Standard Operating Procedures for Freshwater Drift Collection, Processing, and Analysis

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SIGNATURES ON FILE

Please note that the Washington State Department of Ecology's Standard Operating Procedures (SOPs) are adapted from published methods, or developed by in-house technical and administrative experts. Their primary purpose is for internal Ecology use, although sampling and administrative SOPs may have a wider utility. Our SOPs do not supplant official published methods. Distribution of these SOPs does not constitute an endorsement of a particular procedure or method.

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Although Ecology follows the SOP in most instances, there may be instances in which Ecology uses an alternative methodology, procedure, or process.

SOP Revision History

Revision Date	Rev number	Summary of changes	Sections	Reviser(s)
02/26/2007	1.1	Detritus component categories	6.2.6	S. Estrella
08/06/2007	1.2	Stable isotope sample weights	6.3.5, 6.3.7	S. Estrella
05/05/2008	2.0	Updated equipment list and summary of procedures; added subsampling section (6.3); added sample datasheets (11.0)	5.0, 6.0, 11.0	S. Estrella
02/24/2016	2.0	Recertified	All	B. Kammin

Environmental Assessment Program

Standard Operating Procedure for Freshwater Drift Collection, Processing, and Analysis

1.0 Purpose and Scope

- 1.1 This document is the Environmental Assessment Program Standard Operating Procedure (SOP) for collecting, processing, and analyzing freshwater drift samples.
- 1.2 Freshwater drift sampling uses drift nets deployed at a specified point in a stream over a specified period of time to collect macroinvertebrates and detritus transported downstream with stream flow. Sampling typically occurs over a 24-hour period to capture diel changes in drift, as macroinvertebrates are more likely to drift at night. Drift sampling may be used to quantify macroinvertebrate and detrital production in a stream over time or in response to ecosystem disturbances. Macroinvertebrates and detritus are important food sources, and changes in drift quantities may have important implications for downstream fish and amphibian communities.

2.0 Applicability

- 2.1 This document was developed as a freshwater drift sample collection, processing, and analysis procedure for the Type N Experimental Buffer Treatment (Type N) Study. The procedure may be applicable for other studies assessing the transport of detritus and macroinvertebrates in freshwater streams in terms of dry mass and density.
- 2.2 Drift density calculations are based on the volume of water sampled during drift net deployment. For this study, estimates of stream flow through the drift net are based on hydrological measuring devices installed in the Type N study basins. If such devices are not available for other studies, the investigator will have to use another means with which to sample stream flow.
- 2.3 Sample processing for stable N and C isotope analysis is a requirement for some of the study basins. This procedure may or may not be useful for other studies.

3.0 Definitions

- 3.1 Detritus: decomposing organic material consisting of leaves, needles, wood, and other matter.
- 3.2 Drift: stream organisms and organic material transported downstream with stream flow.
- 3.3 Macroinvertebrates: non-microscopic invertebrates that inhabit the stream benthos or enter the stream from the terrestrial environment.
- 3.4 Type N: perennial and seasonal non fish-bearing streams under Washington State's current stream typing system (WAC 222-16-030).

4.0 Personnel Qualifications/Responsibilities

- 4.1 Knowledge of the contents of this SOP.
- 4.2 Ability to use taxonomic keys to identify macroinvertebrates.
- 4.3 Previous experience with macroinvertebrate identification is required for sample processing for stable isotope analysis as live organisms need to be quickly identified before freezing.

5.0 Equipment, Reagents, and Supplies

- 5.1 250 µm drift net
- 5.2 Watch
- 5.3 Waterproof datasheets or data book
- 5.4 Pencil
- 5.5 4 L square polyethylene jar
- 5.6 Labels
- 5.7 Permanent marker
- 5.8 70 percent ethanol—prepared by diluting 100 percent ethanol to 70 parts ethanol and 30 parts deionized water; ethanol that is not being actively used should be stored in the flammables storage building at the Department of Ecology Headquarters; contact with ethanol in the field and laboratory should be minimal
- 5.9 Cooler—size and quantity determined by number of samples collected per day; cooler must be able to accommodate 4 L square polyethylene jars
- 5.10 Ice—quantity determined by number of samples collected per day; must be enough to cover sample jars
- 5.11 1 mm sieve
- 5.12 250 µm sieve
- 5.13 Dissecting scope—Unitron ZSB or equivalent; 10x eyepiece; 0.7x to 4.5x zoom range
- 5.14 Petri dish
- 5.15 Stainless steel forceps
- 5.16 Teasing needles
- 5.17 Vials
- 5.18 Paper bags
- 5.19 Macroinvertebrate identification keys
- 5.20 Ruler
- 5.21 Counting tray

- 5.22 Drying oven—Thelco GCA/Precision Scientific, VWR gravity convection oven, or equivalent
- 5.23 Oven thermometer—50 to 300°C (100 to 600°F) range
- 5.24 Plastic bags—Ziploc bags or equivalent; large enough to contain the sample
- 5.25 Silica drying agent
- 5.26 Scale—Mettler or equivalent; 0.1 mg resolution
- 5.27 Crucible
- 5.28 Weighing dish
- 5.29 Scoop
- 5.30 Muffle furnace—Barnstead/Thermolyne or equivalent
- 5.31 Crucible tongs
- 5.32 Silicone mat
- 5.33 Refrigerator—large enough to contain the samples
- 5.34 Aluminum foil
- 5.35 Freezer—large enough to contain the samples

6.0 Summary of Procedure

- 6.1 Sample Collection
 - 6.1.1 Install a 250 µm mesh drift net at the downstream end of the study area. Place the drift net in the stream with the net opening facing upstream.
 - 6.1.2 Record the date and time of net installation onto a waterproof datasheet or data book using a pencil. Note the percent flow volume sampled by the drift net.
 - 6.1.3 Avoid walking upstream of the drift net during drift net deployment.
 - 6.1.4 Return to the study area 24 hours later at approximately the same time. Logistics and stream flows may necessitate sample collection over a longer or shorter period in some streams, in which case the data will need to be adjusted.
 - 6.1.5 Remove the drift net from the stream and record the date and time of net removal. Note the percent flow volume sampled by the drift net.
 - 6.1.6 Empty the contents of the drift net into a 4 L square polyethylene jar. Label the jar with the study name, site identifier, and sample collection date and time using a permanent marker.
 - 6.1.7 If the sample is not being used for stable isotope analysis, preserve the sample in 70 percent ethanol.
 - 6.1.8 If the sample is being used for stable isotope analysis, DO NOT preserve the sample with ethanol. Store the sample in a cooler on ice.
 - 6.1.9 Repeat the sampling procedure as dictated by the experimental design.

- 6.2 Sample Processing
- 6.2.1 Sieve the drift sample through 1 mm and 250 μm nested sieves.
- 6.2.2 Examine the contents of each sieve for macroinvertebrates using a scope, petri dish, forceps, and teasing needles.
- 6.2.3 Log the sample onto a lab datasheet (see Litterfall Sorting Form in Section 11.0).
- 6.2.4 Separate the macroinvertebrates from the detritus and store in labeled vials containing 70 percent ethanol. Vials should be labeled with the study name, site identifier, sample collection date, and variable (“macroinvertebrates”).
- 6.2.5 Designate the detritus retained on the 1 mm sieve as coarse particulate organic matter (CPOM) (after Melody and Richardson 2004). Place the detritus sample in a paper bag labeled with the study name, site identifier, sample collection date, variable (“detritus”), and CPOM. Allow the sample to air dry for a couple of days (A. Foster, USFS, personal communication).
- 6.2.6 Designate the detritus retained on the 250 μm sieve as fine particulate organic matter (FPOM) (after Melody and Richardson 2004). Place the sample in a paper bag labeled with the study name, site identifier, sample collection date, variable, and FPOM. Allow the sample to air dry for a couple of days.
- 6.2.7 Identify all of the macroinvertebrates in the sample to the lowest practical taxonomic level as designated in Plotnikoff and White (1996) using identification keys.
- 6.2.8 Measure macroinvertebrate body lengths to the nearest 1 mm using a ruler.
- 6.2.9 Record macroinvertebrate taxon name and length.
- 6.2.10 Store identified macroinvertebrates in vials with 70 percent ethanol.
- 6.2.11 Sort the CPOM detritus sample into deciduous leaves, coniferous needles, woody material (twigs, branches, bark, cones, etc.), and miscellaneous (bud scales, etc.) components on the counting tray using forceps and teasing needles. Different size sieves may be helpful in sorting the components.
- 6.2.12 Place each component in a paper bag labeled with the study name, site identifier, sample collection date, variable (“detritus”), and component (e.g. “CPOM-coniferous”).
- 6.2.13 Dry each CPOM sample component (deciduous leaves, coniferous needles, woody material, miscellaneous) and the FPOM sample in the paper bag in the drying oven at 55 degrees C for 96 hours. Use an oven thermometer to check the temperature.
- 6.2.14 Cool each detritus component in a dry plastic Ziploc bag or equivalent with a silica drying agent (A. Foster, USFS, personal communication). Label the bag with the study name, site identifier, sample collection date, variable (“detritus”), and component (e.g. “CPOM-coniferous”). Store the sample in the plastic bag until the sample is weighed and ashed.

- 6.2.15 Weigh the entire sample on a calibrated scale using a crucible, weighing dish, or other container to hold the sample. Record as dry weight (see Detritus Sample Processing Form in Section 11.0).
- 6.2.16 If the entire sample is larger than about one-third to one-half of the capacity of the crucible, it is necessary to subsample the total sample before ashing. To subsample, thoroughly mix the sample in a large container or tub with a scoop. Use forceps or a scoop to remove enough sample to fill about one-third to one-half of the crucible's capacity. Compare the subsample to the rest of the sample to ensure that the subsample is representative of the entire sample.
- 6.2.17 Weigh the subsampled portion in the crucible prior to ashing and record as subsample dry weight.
- 6.2.18 Ash each component in a crucible in the muffle furnace at 550 degrees C for at least one hour. Use crucible tongs to add and remove crucibles to and from the furnace.
- 6.2.19 Cool each detritus component on a silicone mat.
- 6.2.20 Weigh each component and record as ashed weight.

6.3 Subsampling and Processing Large Detritus Samples

- 6.3.1 After removing macroinvertebrates from the detritus sample, place the detritus in a paper bag labeled with the study name, site identifier, sample collection date, and variable ("detritus").
- 6.3.2 Dry the entire detritus sample in a paper bag in the drying oven at 55 degrees C for at least 96 hours. Use an oven thermometer to check the temperature.
- 6.3.3 Cool the detritus sample.
- 6.3.4 Weigh the entire sample on a calibrated scale using a weighing dish or other container to hold the sample. Record as total sample dry weight (see Subsampled Detritus Sample Processing Form in Section 11.0).
- 6.3.5 To subsample, thoroughly mix the sample in a large container or tub with a scoop. Use a scoop to remove enough sample that is reasonable to sort within one to two days. Compare the subsample to the rest of the sample to ensure that the subsample is representative of the entire sample.
- 6.3.6 Weigh the subsampled portion and record as total subsampled dry weight.
- 6.3.7 Log the sample onto a lab datasheet (see Drift Sorting Form in Section 11.0).
- 6.3.8 Sort the subsampled detritus sample into deciduous leaves, coniferous needles, woody material (twigs, branches, bark, cones, etc.), and miscellaneous (bud scales, etc.) components on the counting tray using forceps and teasing needles. Different size sieves may be helpful in sorting the components.
- 6.3.9 Place each component in a paper bag labeled with the study name, site identifier, sample collection date, variable ("detritus"), and component (e.g. "coniferous").

- 6.3.10 Dry each component in the paper bag in the drying oven at 55 degrees C for at least 96 hours. Use an oven thermometer to check the oven temperature.
- 6.3.11 Cool each detritus component in a dry plastic Ziploc bag or equivalent with a silica drying agent (A. Foster, USFS, personal communication). Label the bag with the study name, site identifier, sample collection date, variable (“detritus”), and component (e.g. “coniferous”). Store the sample in the plastic bag until the sample is weighed and ashed.
- 6.3.12 Weigh the sample on a calibrated scale using a crucible, weighing dish, or other container to hold the sample. Record as component dry weight (see Subsampled Detritus Sample Processing Form in Section 11.0).
- 6.3.13 If the sample is larger than about one-third to one-half of the capacity of the crucible, it is necessary to subsample the sample before ashing. To subsample, thoroughly mix the sample in a large container or tub with a scoop. Use forceps or a scoop to remove enough sample to fill about one-third to one-half of the crucible’s capacity. Compare the subsample to the rest of the sample to ensure that the subsample is representative of the sample.
- 6.3.14 Weigh the subsampled portion in the crucible prior to ashing and record as component subsample dry weight.
- 6.3.15 Ash each component in a crucible in the muffle furnace at 550 degrees C for at least one hour. Use crucible tongs to add and remove crucibles to and from the furnace.
- 6.3.16 Cool each detritus component on a silicone mat.
- 6.3.17 Weigh each component and record as component ashed weight.

6.4 Sample Processing for Stable N and C Isotope Analysis

- 6.4.1 Store the drift samples in a refrigerator for at least 24 hours to allow the macroinvertebrates to evacuate the gut (B. Bilby, Weyerhaeuser Company, personal communication).
- 6.4.2 Sieve the drift sample through 1 mm and 250 µm nested sieves.
- 6.4.3 Examine the contents of each sieve for macroinvertebrates using a scope, petri dish, forceps, and teasing needles.
- 6.4.4 Separate the macroinvertebrates from the detritus and store in water in labeled vials. Vials should be labeled with the study name, site identifier, sample collection date, and variable (“macroinvertebrates”). Refrigerate the sample until identification. Macroinvertebrates should be identified within one to two weeks.
- 6.4.5 Recombine the detritus sample from the sieves, thoroughly mix, and remove two to three milligrams dry weight of sample from the total sample. Record the weight removed (see Drift Sorting Form).
- 6.4.6 Wrap the sample with aluminum foil and bag the sample in a plastic bag (B. Bilby, Weyerhaeuser Company, personal communication).

- 6.4.7 Look through the macroinvertebrate sample in the petri dish and identify the two most abundant macroinvertebrate taxa using a macroinvertebrate identification key (B. Bilby, Weyerhaeuser Company, personal communication). For each the two taxa, select ~500 mg wet weight of sample. If there is not enough mass, include the entire amount collected.
- 6.4.8 Measure the body lengths of the selected macroinvertebrates to the nearest 1 mm with a ruler.
- 6.4.9 Record the taxon and lengths of the selected macroinvertebrates.
- 6.4.10 Store the selected macroinvertebrates by taxon in a vial filled with water. Freeze the sample. Wrap the vials with aluminum foil and bag the samples in a plastic bag.
- 6.4.11 Label the plastic bags containing the detritus and macroinvertebrate samples with the study name, site identifier, sample collection date, variable (“detritus” or “macroinvertebrates”), and taxon (e.g. “*Baetis*”).
- 6.4.12 Freeze the samples and ship to the sample processor.
- 6.4.13 Detritus samples not used in the stable isotope analysis should be processed according to the procedures outlined in Sections 6.2 or 6.3.
- 6.4.14 Macroinvertebrate samples not used in the stable isotope analysis should be preserved in 70 percent ethanol and processed according to the procedure in Section 6.2.

6.5 Sample Analysis

- 6.4.1 Estimate the total volume of water passing through the drift net during deployment from discharge measurements obtained by the hydrological measuring devices.
- 6.4.2 Adjust the data to reflect a 24-hour drift net deployment period and 100 percent flow volume.
- 6.4.3 Calculate detritus transport as dry mass (g) per day by each detritus component and density per day by each detritus component. Density is calculated as the dry weight of each detritus category collected in the stream divided by the volume of water sampled.
- 6.4.4 Use measured length of macroinvertebrates and taxon-specific length:dry mass regression equations in Rogers et al. (1977), Smock (1980), Meyer (1989), Sample et al. (1993), and Burgherr and Meyer (1997) to estimate macroinvertebrate dry mass (after Wipfli and Gregovich 2002, Musslewhite and Wipfli 2004).
- 6.4.5 Assign macroinvertebrates to functional feeding groups after Merritt and Cummins (1996) and Wisseman (1998).
- 6.4.6 Calculate macroinvertebrate transport as numbers per day, dry mass (mg) per day, and density per day by taxa and functional feeding group. Density is calculated as the total weight of each taxa and functional feeding group collected in the stream divided by the volume of water sampled.
- 6.4.7 Calculate relative abundance of macroinvertebrate taxa and functional feeding groups.

7.0 Records Management

7.1 Enter data into an Access database.

8.0 Quality Control and Quality Assurance

8.1 Avoid wading or conducting other measurements upstream of the drift net during deployment.

8.2 Ensure that datasheets are completely filled out in the field.

8.3 Ensure that sample collection jars are tightly sealed and correctly labeled.

8.4 Ensure that all paper bags, plastic bags, and crucibles used in sample processing are correctly labeled.

8.5 Re-identify five percent of the macroinvertebrate samples collected for each year of the study. Errors in identification should be less than five percent of the total macroinvertebrate taxa in the sample (Plotnikoff and Wiseman 2001).

8.6 Check all data entered into the database for accuracy and completeness.

9.0 Safety

9.1 File a field work plan before commencing field activities.

9.2 Use a CB radio to communicate with other traffic on one-way logging roads.

9.3 Learn how to deal with animals and people encountered in remote areas.

9.4 Store large supplies of ethanol in the flammables storage building at the Department of Ecology Headquarters.

9.5 Minimize contact with ethanol in the field and laboratory.

10.0 References

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11.0 Sample Datasheets

- 11.1 X:\EA PROGRAM\ECYEAPSOP\Approved SOPs\Type N Experimental Buffer Study Attachments\TypeNExperimentalBufferSOP_datasheets.xls