Washington State Department of Ecology

Environmental Assessment Program

# Standard Operating Procedures for the Collection and Processing of Samples for Stable Isotope Analysis

Version 1.0

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*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.*

*Any reference to specific equipment, manufacturer, or supplies is for descriptive purposes only and does not constitute an endorsement of a particular product or service by the author or by the Department of Ecology.*

*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) |
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Environmental Assessment Program

Standard Operating Procedures for the Collection and Processing of Samples for Stable Isotope Analysis

## 1.0 Purpose and Scope

1.1 This document is the Environmental Assessment Program Standard Operating Procedure (SOP) for the collection and processing of samples for stable isotope analysis.

1.2 Benthic invertebrates, leaf litter, and periphyton samples are collected up to four times annually as part of the Intensively Monitored Watersheds (IMW) project in the complexes where treatment streams receive carcass analog applications. They are analyzed for stable 15N and 13C isotopes both before and after carcass analog application. These data illuminate patterns of nutrient cycling through different trophic levels in the riparian ecosystem to determine what effect carcass analogs have compared to untreated streams. This SOP covers only collection and preparation of samples for shipping. Actual lab analysis and quantification of stable isotopes is conducted subsequently by a lab that specializes in that procedure.

## 2.0 Applicability

2.1 This document was developed for use in the IMW Lower Columbia and Strait of Juan de Fuca complex streams. Because the samples are collected and processed for stable isotope analysis, this procedure may or may not be useful, in whole or in part, for other studies.

2.2 The interest in collecting stable isotope samples lies in collecting and analyzing tissue for stable isotopes, not in quantifying periphyton, leaf litter, and macroinvertebrates or describing community structure. As such, other similar SOPs on the subject of periphyton, leaf litter, and macroinvertebrate sampling are more labor intensive and stringent than is required for these sampling purposes. Similarly, the guidance outlined here will not be sufficient if the goals of sampling are to quantify individuals or describe community structure.

## 3.0 Definitions

3.1 Benthic macroinvertebrates: non-microscopic invertebrates that inhabit the stream benthos or enter the stream from a terrestrial environment (EAP SOP #016).

3.2 Periphyton: benthic algae that grow attached to surfaces such as rocks.

3.3 Stable isotope analysis: an analysis to determine the identification and distribution of stable forms of C and N that can be used to make inferences on diet and trophic levels in aquatic ecosystems.

3.4 Kick net: a net attached to a D-ring shaped frame with a long handle, canvas sides, and a 500 micron mesh back.

## 4.0 Personnel Qualifications/Responsibilities

4.1 Knowledge of the contents of this SOP.

4.2 Knowledge of the contents of the EAP Safety Manual Chapters 1 and 2.

## 5.0 Equipment, Reagents, and Supplies

5.1 4 L square polyethylene jars (one per site)

5.2 D-frame kick net with 500 micron mesh back

5.3 125 mL plastic sample containers with lids (one per site)

5.4 Small scrub brush

5.5 500 mL wide mouth plastic jar

5.6 Aluminum foil

5.7 Plastic “sandwich” bags

5.8 Cooler(s)

5.9 Ice

5.10 Grease pencil and permanent marker

5.11 1mm and 500 micron nested sieves

5.12 Microscope

5.13 Petri dish

5.14 Forceps

5.15 Teasing needle

5.16 Glass vials

5.17 Label tape

5.18 Macroinvertebrate identification keys

5.19 Wash bottle

## 6.0 Summary of Procedure

6.1 <u>Periphyton</u>

6.1.1 Identify location of sampling site.

6.1.2 Fill a 500 mL wide mouth plastic jar with a small amount of water.

6.1.3 Scrub periphyton off the surface of small rocks (up to the size of a computer mouse) using a small scrub brush, occasionally dunking the rock and the brush in the jar of water to rinse.

6.1.4 When a sufficient quantity of periphyton has been collected, pour the water and periphyton mixture from the jar into a 125 mL sampling container, swirling gently so that any periphyton that has settled to the bottom of the jar is mixed back into the water and transferred to the container. Leave at least half an inch of headspace to accommodate expansion from freezing.

6.1.5 Label the jar lid with the project name, stream name and site number, variable (*Periphyton*), and the date. Store on ice.

6.2 Alder Leaves

6.2.1 Identify location of sampling site.

6.2.2 Collect one alder leaf from a tree.

6.2.3 Fold the leaf in aluminum foil.

6.2.4 Label the foil with the project name, stream name and site number, variable (*Alder leaf*), and the date.

6.2.5 Slip the enclosed leaf in a plastic "sandwich" bag and store on ice.

6.3 Macroinvertebrates

6.3.1 Identify a riffle in the sampling reach.

6.3.2 Rinse a 4 L square polyethylene sampling jar three times with stream water. Fill the jar up to halfway with stream water.

6.3.3 Place the flat side of the D-ring kick net on the bottom of the streambed.

6.3.4 Kick the substrate in front of the net vigorously. For small substrate reaches (silt, sand, gravel) two kick repetitions may be sufficient. For large substrate reaches (cobble, boulders), repeat this procedure again for a third or a fourth kick repetition. The number of kick repetitions is less important than the quantity of sample collected. Kick the sediment for as long and as repeatedly as is necessary to acquire a sample roughly the size of a baseball. If necessary, move the kick net to a new location in the stream at the same site to increase the sample size.

6.3.7 Composite the samples at each site into one 4 L square polyethylene jar by sweeping the net against the current and consolidating the sample into a single mass. Inspect the mass and remove crayfish and stoneflies to minimize predation.

6.3.8 Dump the sample mass into the container and use a wash bottle to flush remaining material from the net into the container.

6.3.9 Label the container lid with project name, stream name and site number, variable (*Macroinvertebrates*), and date.

6.3.10 Store on ice.

6.4 Sample Management

6.4.1 Keep samples on ice until they are returned to the OC. Replenish ice as needed.

6.4.2 Upon returning to the OC, label sample coolers with a tag that indicates they are not for Manchester Environmental Laboratory pickup. Place them in the OC walk-in cooler.

6.5 Sample Processing

6.5.1 Freeze alder leaves and periphyton and ship to Weyerhaeuser.

6.5.2 Store the macroinvertebrates on ice or in a refrigerator for at least 24 hours to allow the specimens to evacuate the gut.

6.5.3 Sieve the sample through 1 mm and 500 micron nested sieves.

6.5.4 Examine the contents of each sieve for macroinvertberates using a scope, petri dish, forceps, and teasing needles.

6.5.5 Separate the macroinvertebrates from the detritus and store in water in labeled vials. Vials should be labeled with study name, stream name and site number, variable (*Macroinvertebrates*), and collection date. Refrigerate the sample until identification.

6.5.6 Identify macroinvertebrates to Family using a taxonomic key. For guidance on keying specimens, see the SOP for Freshwater Drift Collection, Processing, and Analysis (EAP SOP #016).

6.5.7 Determine the two most abundant taxa representing the scraper and shredder feeding groups. For each taxon, select enough individuals to satisfy the biomass minimum for analysis (refer to contracting lab for necessary biomass). If there is not enough mass, use the entire amount collected. Additional abundant taxa representing other feeding groups, such as collectors, may also be submitted.

6.5.8 Store the selected macroinvertebrates by taxon in a vial filled with water. Freeze the sample. Bag the samples in a plastic “sandwich” bag. Label the bags with study name, stream name and site number, variable (*Macroinvertebrates*), collection date, and taxon.

6.5.9 Freeze the samples and ship to Weyerhaeuser with periphyton and alder leaves for further processing and shipment to an analytical lab.

## 7.0 Records Management

7.1 Maintain a detailed manifest of sample collection and processing. Save the manifest to a network drive that is backed up nightly.

7.2 Include the manifest each time samples change custody. Consolidate manifests from other entities if samples change custody several times, and maintain change of custody records within the manifests. Save consolidated manifests to a network drive that is backed up nightly.

7.3 After results are returned from the contract lab, store them in the same location as manifests, on a network drive that is backed up nightly. Results should be in an electronic format compatible with the software used for statistical analyses (Excel workbook or Access database). If necessary, export the relevant data from the raw results returned from the contract lab to a useable format.

## 8.0 Quality Control and Quality Assurance

8.1 Correctly label all sample jars immediately upon collection.

8.2 Ensure that sample jars are tightly sealed.

8.3 Ensure accuracy and completion of sample manifest.

8.4 Replenish ice as needed to prevent decomposition of samples and macroinvertebrate cannibalism.

## 9.0 Safety

9.1 File a field work plan before commencing field activities.

9.2 Use caution when walking in stream channels and while wearing waders. See EAP Safety Manual Chapter 1: General Field Work – Working in Rivers or Streams. Consider wearing a PFD in deep water. The use of a PFD is mandatory where the danger of drowning exists.

9.3 Use caution when lifting ice and sample filled coolers. See Ecology Safety Program Manual – Lifting Safety.

9.4 If sampling is concurrent with Weyerhaeuser electrofishing, follow all guidance in the EAP Safety Manual Chapter 2: Specialized Work – Sampling with Battery Powered Backpack Electrofishers.

## 10.0 References

10.1 Environmental Assessment Program. 2012. Environmental Assessment Program Safety Manual. Washington State Department of Ecology, Olympia, WA.

10.2 Estrella, S. 2008. Standard Operating Procedure (SOP) for Freshwater Drift Collection, Processing, and Analysis, v. 2. Washington State Department of Ecology, Olympia, WA. http://www.ecy.wa.gov/programs/eap/qa/docs/ECY_EAP_SOP_FreshwaterDrift_v_2_0EAP016.pdf

10.3 "Lifting Safety." 2008. Ecology Safety Program Manual. Washington State Department of Ecology, Olympia, WA. http://aww.ecology/services/es/Safety/Safety_Web/lifting.htm