Macroinvertebrate Drift Sample Processing Standard Operating Procedure

Draft

Prepared for

the Columbia Habitat Monitoring Program

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Sample Arrival and Inventory

Upon arrival of each sample batch, jars will be examined for sample condition and checked against accompanying inventory/custody documents and sample identifier lists. Samples arriving decanted will be reconstituted with a volume of 95% ethanol sufficient to fully cover the contents of the jar. Drift collections assigned identical DCeNames and Site IDs along with "Net Number 1" and "Net Number 2" on the inventory/custody documents will be combined together into a single sample for analysis. The information from each inventory/custody document will be formatted for upload to the CHaMP database as well as to Rhithron's laboratory information system. Internal project and sample identifiers will be assigned to each individual sample for tracking in the Rhithron laboratory.

Sample Processing

Standard Size Sample Processing Procedure:

Each sample will be poured out, rinsed, and thoroughly mixed in a no. 35 standard sieve (18 inch diameter, 500µm). Sample material is gently rinsed with low-pressure water spray to remove sand and silt. Sample material will then be transferred and evenly distributed into a Caton subsampling tray. The sieve is examined under lighted magnification to ensure retrieval of all organisms. Caton trays (Caton 1991) are divided into 30 grids (each approximately 6 cm by 6 cm), and facilitate the standardization of grids. A single grid is randomly selected, and the contents of the grid will be transferred to a petri dish. This material will be examined under stereoscopic microscopes using 10x-35x magnification (Leica S6E and Leica EZ4). All organisms from each selected grid will be sorted from the substrate and placed in vials with 80% ethanol. Grid selection, examination, and sorting will continue until a subsample of at least 600 organisms is obtained, or until the sample is completely sorted. The final grid will be completely sorted of all organisms.

Technicians record the total number of grids sorted.

Large Size Sample Processing Procedure:

Large size samples are those that occupy more than 1.5cm depth in a single standard Caton tray. Each large size sample is completely poured out, rinsed, and thoroughly mixed in a no. 35 standard sieve (18 inch diameter, 500µm). Sample material is gently rinsed with low-pressure water spray to remove sand and silt. Sample material will then be transferred and evenly distributed into a 50.8 cm^2 splitting tray. The sieve is examined under lighted magnification to ensure retrieval of all organisms. The splitting tray is divided into four quadrants. Sample contents are evenly distributed in the splitting tray.

A single quadrant is randomly selected, and sample material is lifted from the splitting tray into a Caton tray. Sample material is evenly distributed in the Caton tray. Standard subsampling techniques are then applied (detailed above). Standard-sized grids (each approximately 6 cm by 6 cm) are randomly selected, and the contents of the grid will be transferred to a petri dish. All organisms are sorted from each grid, under stereoscopic microscopes using 10x-35x magnification (Leica S6E and Leica EZ4).

If all of the sample material in the Caton tray is sorted before the target number of organisms is achieved, a second quadrant from the splitting tray is randomly selected, and sorting continues in this manner until the target number of organisms is reached, or until 2 quadrants are entirely sorted.

Technicians record the total number of grids sorted.

The Large Organism Search

If the target number of organisms is reached before the entire sample is sorted, a 15-minute Large-Organism search will be performed on the unsorted sample material remaining in the Caton tray and/or splitting tray. The search will be focused on organisms 20mm or more in length, and ALL such organisms will be sorted from the unsorted substrate. Large Organisms will be placed in a separate labeled vial and will be delivered to the taxonomy department along with the subsample.

Quality Control / Quality Assurance

Quality control procedures for initial sample processing and subsampling involve checking sorting efficiency. These checks will be conducted on a minimum of 10% of samples: quality control samples are randomly selected. Quality assurance technicians, who have received additional training, testing, and certification, microscopically re-examine all sorted substrate from each quality control sample. Quality control procedures for each selected sample will proceed as follows:

The quality assurance technician will pour the sorted substrate from a processed sample out into a Caton tray, redistributing the substrate in a similar fashion as the primary technician. All organisms that are missed are counted and this number is added to the total number obtained in the original sort. Sorting efficiency is calculated by applying the following calculation:

$$SE = \frac{n_1}{n_2} \times 100$$

where: SE is sorting efficiency, expressed as a percentage; n_1 is the total number of specimens in the first sort; and n_2 is the total number of specimens in the first and second sorts.

If 90% sorting efficiency is not achieved for a given sample, a failure will be recorded in the database. The sorted fraction of that sample will then be completely resorted before the sorting efficiency test is repeated for that sample. Sorting efficiency for each randomly selected sample in the project will be reported to the client.

Taxonomic Analysis

Identification

Organisms will be individually examined by certified taxonomists, using 10x – 80x stereoscopic dissecting scopes (Leica S8E) and categorized (Aquatic organisms, Emergent Adults, and Terrestrial organisms), life stage (pupa, larva, adult, unknown) and size class. Organisms will be identified following the taxonomic resolution listed in Table 1, using appropriate published taxonomic references and keys.

Morphometry

Total body length of each organism will be measured using ocular or stage micrometers. Each organism will be assigned to a size class. Size classes are: ≤3mm, >3mm but ≤6mm, >6mm but ≤9mm, and so on, each class defined by a 3mm length range. If an organism is damaged so that an accurate size class cannot be measured, the total length of the specimen is estimated using other individuals of the same taxon in similar stages of maturity or similar instars from within the sample. If no similar individuals of the same are present in the sample, size class is estimated by best professional judgment.

Diet Component Resource Classes

Organisms are also identified by diet component resource classes for dry mass determination. These classes are defined as: 1) Aquatic organisms 2) Emergent adults and 3) Terrestrial organisms. Aquatic organisms are those individuals and life stages that would be identified in a standard bioassessment sample. Emergent adults are individuals and life stages that have aquatic origins but are generally excluded from standard bioassessment samples. (e.g. adult mayflies, stoneflies and caddisflies, and some adult dipterans). Terrestrial organisms are those individuals and life stages without aquatic origins. Table 2 is a guide to the diet component resource classes of life stages and taxa.

Quality Control /Quality Assurance

Quality control (QC) procedures for taxonomic determinations of invertebrates involve checking accuracy, precision and enumeration. Ten percent of samples in the project will be randomly selected and all organisms re-identified and counted by an independent taxonomist. Taxa lists and enumerations will be compared by calculating 2 evaluative parameters: the Percent Taxonomic Disagreement (PTD) and the Percent Difference in Enumeration (PDE) (Stribling et al. 2003) for each selected sample.

Taxonomic disagreements between the original identifications and the QC identifications are discussed among the taxonomists, and necessary rectifications to the data are made.

Samples which have ≥10% PTD or ≥5% PDE will be regarded as QC failures. Each failure will trigger another sample quality check. QC parameters for each randomly selected sample in the project will be reported to the client.

Large Organism Fraction Procedure

After the taxonomist has identified, counted, and classed all organisms in the subsample, the Large Organism portion is similarly identified, counted, and classed. In the data, the Large Organism portion is distinguished from the subsample portion by appending "Large" to the diet component resource class designation.

Biomass Determination

Filters (47mm glass, Whitman Glass –Fiber Filters Type GF/A, 1.6 micron pore size) will be placed in numbered aluminum boats and pre-ashed by heating at 500º C for 20 minutes. Boats and filters will be placed in a desiccation chamber, allowed to cool to room temperature and weighed to the nearest 0.1 milligram using a calibrated precision balance (Acculab ALC-210.4). Each filter will be placed in a filtration apparatus and moistened with 2 mL of de-ionized water before the organisms are added. Each sample is represented by up to 3 portions, based on diet resource classes (Aquatic organisms, Emergent adults, or Terrestrial organisms). Each sample portion will be thoroughly mixed and filtered and each vial will be rinsed twice with 10 mL of de-ionized water. The filtering apparatus will be given a final rinse with 10 mL of de-ionized water.

The filters will then be placed back in their numbered boats and dried to a constant weight at 105° C in a drying oven. Each sample portion is dried at 105° C for a minimum of 2 hours. Sample fractions containing large taxa, or taxa that are difficult to dry (e.g. crayfish or salmonflies) are dried for additional 1 hour intervals, with weights taken after each additional hour, until no significant change in mass (±0.0005g) is measured from one weighing to another. Weights for

each sample portion will be recorded to the nearest 0.1 milligram.

The Large Organism portion is dried and weighed by diet resource classes, separately from the subsample, using identical methods.

Quality Assurance / Quality Control:

At the beginning of each weighing session, the Acculab ALC-210.4, precision balance will be calibrated using a certified 200-gram weight, using procedures recommended by the manufacturer.

Each batch of 20 biomass fractions will include a laboratory blank. Summary statistics of laboratory blank measures will be reported to the client.

Data Management

Rhithron will report data to the CHaMP project database in the required data exchange format. The exchange format will include four files of comma-separated values (csv). The four files are:

- Taxonomic.csv – metadata table that defines taxonomic codes including ITIS serial number, taxonomic hierarchy (phylum to species), life stage, and any available information on functional feeding groups, habitat niche, and environmental tolerances.
- Sample.csv – metadata table that describes the sample, including: site id, sample date and time, replicate number, sample jar, sorting technician, sorting time, and portion of sample sorted.
- TaxonBySizeClassCount.csv – count data resulting from sample sorting. Data will include: sample identifier, taxonomic identification, size class, and observed count.
- SampleBiomass.csv. – dry weight biomass of drift sample portion. Data will include: sample identifier, resource class, tare weight, and measured dry weight.

Table 1. Taxonomic resolution targets for CHaMP drift organisms. Current nomenclature will be reported and taxa translation tables provided as needed.

Taxonomic Order	Level of Identification
Copepoda	Copepoda (Subclass)
Ostracoda	Ostracoda (Class)
Diplostraca	Cladocera (Suborder)
Oligochaeta	Oligochaeta (Subclass)
Basommatophora	Genus (Aquatic organisms); Class (Terrestrial organisms)
Neotaenioglossa	Genus (Aquatic organisms); Class (Terrestrial organisms)
Stylommatophora	Genus (Aquatic organisms); Class (Terrestrial organisms)
Veneroida	Genus (Aquatic organisms); Class (Terrestrial organisms)
Hydrozoa	Hydrozoa (Class)
Nematoda	Nemata (Phylum)
Turbellaria	Trepaxonemata (Class)
Amphipoda	Genus
Isopoda	Genus (Aquatic organisms); Order (Terrestrial organisms)
Hirudinea	Genus
Araneae	Araneae (Order)
Opiliones	Opiliones (Order)
Coleoptera	Genus (Aquatic organisms and Emergent Adults); Order (Terrestrial organisms)
Collembola	Order
Diptera	Genus (Aquatic organisms); Order (Terrestrial organisms)
Chironomidae	Subfamily / tribe (Aquatic larvae); Family (Aquatic pupae and Emergent Adults)
Ephemeroptera	Genus (Aquatic Larvae); Family (Emergent Adults)
Hemiptera	Genus (Aquatic organisms); Order (Terrestrial organisms)
Hymenoptera	Order
Lepidoptera	Genus (Aquatic organisms); Order (Terrestrial organisms)
Megaloptera	Genus (Aquatic Larvae); Family (Emergent Adults)
Odonata	Genus (Aquatic Larvae); Family (Emergent Adults)
Plecoptera	Genus (Aquatic Larvae); Family (Emergent Adults)
Psocoptera	Order
Acari	Genus (Aquatic organisms); subclass (Terrestrial organisms)
Thysanoptera	Order

Table 2. Representative organisms in diet component resource classes.

Resource class	Representative organisms
Aquatic organisms	*Larval stages* of Ephemeroptera, Plecoptera, Chironomidae, Odonata. *Larval and pupal stages* of Trichoptera, Tipulidae, Deuterophlebiidae, Psychodidae, Dixidae, Culicidae, Thaumaleidae, Simuliidae, Ceratopogonidae, Athericidae, Statiomyidae, Empididae, Dolichopodidae, Ephydridae. *Larval and adult stages* of Elmidae, Dytiscidae, Hydrophilidae, Hydraenidae.
Emergent adults	*Adult stages* of Ephemeroptera, Plecoptera, Trichoptera, Odonata, Chironomidae, Elmidae, Megaloptera, Tipulidae, Deuterophlebiidae, Psychodidae, Dixidae, Culicidae, Thaumaleidae, Simuliidae, Ceratopogonidae, Athericidae, Statiomyidae, Empididae, Dolichopodidae, Ephydridae.
Terrestrial organisms	*All life stages* of Hymenoptera, Thysanoptera, Collembola, some Hemiptera, Dermaptera, Neuroptera, some Diptera, some Coleoptera, Lepidoptera.

Citations

Caton, L. W. 1991. Improving subsampling methods for the EPA's "Rapid Bioassessment" benthic protocols. Bulletin of the North American Benthological Society. 8(3): 317-319.

Stribling, J. B.; S.R. Moulton II, G.T. Lester. 2003. Determining the quality of taxonomic data. Journal of the North American Benthological Society. 22(4)621-631