



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L602

Macroinvertebrate Sorting, Subsampling, and Identification

Standard Operating Procedure

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1. Purpose

This SOP describes the laboratory procedures for sorting, subsampling, and identifying benthic macroinvertebrates (BMI) collected from wadeable streams per [SOP-F610](#). The procedure follows

the California Surface Water Ambient Monitoring Program (SWAMP) laboratory processing protocols to ensure compatibility with statewide bioassessment datasets (Barbour et al., 1999; Ode et al., 2016). Taxonomic identifications are used to calculate biological condition metrics, including the California Stream Condition Index (CSCI) (Mazor et al., 2016).

2. Scope

- **Applies to:** Laboratory processing of ethanol-preserved BMI samples from wadeable streams, including subsampling, sorting, identification to the lowest practical taxonomic level, and enumeration
- **Does not apply to:** Field collection of BMI (see [SOP-F610](#)); marine or estuarine macroinvertebrates; meiofauna (organisms passing through a 500 μm sieve); molecular identification (eDNA metabarcoding)
- **Location:** EA Program laboratory, SGM 133

3. Responsibilities

Role	Responsibility
PI / Lab Manager	Approve taxonomic level targets; arrange external QC
Trained Personnel	Sort, identify, and enumerate organisms per this SOP
New Students	Complete training before independent sorting

4. Definitions

- **Benthic macroinvertebrate (BMI):** An invertebrate organism inhabiting the bottom substrate of streams, lakes, or wetlands that is retained on a 500 μm mesh sieve; includes aquatic insects, crustaceans, worms, mollusks, and other taxa.
- **Fixed count subsample:** A randomly selected subset of a BMI sample from which a fixed number of organisms (e.g., 500) is picked and identified; reduces processing time for large samples while maintaining statistical reliability.
- **Functional feeding group (FFG):** A classification of macroinvertebrates by feeding mode (shredders, collectors-gatherers, collectors-filterers, scrapers/grazers, predators); used to assess stream ecosystem function from invertebrate assemblage data.
- **Qualitative identification:** Determination of taxa present in a sample without quantifying abundances; used for rapid bioassessment and presence/absence monitoring.
- **Quantitative sample:** A sample in which all organisms are counted and identified to calculate density (organisms per unit area or volume); requires accurate recording of sampled area and volume.
- **Taxonomic resolution:** The level of taxonomic identification applied to BMI specimens; options range from phylum/class to order, family, genus, or species; standard SWAMP protocol requires family-level identification for most groups.

- **Voucher specimen:** A representative specimen of a taxon retained in preservative as a reference for future verification; essential for quality assurance and resolving taxonomic uncertainties.

5. Safety and Hazards

Hazards

- **Chemical:** Preserved samples contain approximately 70% ethanol, which is flammable and an inhalation irritant. Work in a well-ventilated area away from ignition sources.
- **Physical:** Forceps and dissecting needles are sharp. Use caution when handling small organisms.
- **Biological:** Stream organisms may harbor parasites or pathogens. Wear gloves when handling preserved samples.

5.1. Required PPE

- ☐ Nitrile gloves
- ☐ Safety glasses
- ☐ Lab coat

5.2. Emergency Procedures

For ethanol spills, ventilate the area, absorb with paper towels, and dispose in flammable waste. Refer to [SOP-L002 Laboratory Safety](#).

6. Equipment and Materials

6.1. Equipment

Item	Notes
Dissecting microscope (stereo)	7–45x magnification; illuminated base
Compound microscope	For slide-mounted specimens requiring higher magnification
Gridded Caton sorting tray	White, subdivided into numbered cells
Fine-tipped forceps (2 pairs)	Dumont #5 or equivalent
Dissecting needles	For separating tangled organisms
Petri dishes	For isolating specimens during ID
Wash bottles (ethanol and DI water)	

6.2. Consumables

Item	Notes
70% ethanol	For topping off vials
Scintillation vials (20 mL)	For archiving sorted taxa
Labels (pencil on waterproof paper)	Internal and external labels
Depression slides	For compound microscope work

7. Procedure

7.1. Sample Receiving and Logging

1. Verify that the sample jar label matches the chain-of-custody or field data sheet: site ID, date, collector, preservative.
2. Log the sample into the laboratory tracking spreadsheet.
3. Inspect the sample for adequate preservative (organisms should not appear soft or discolored). If the ethanol is diluted below approximately 50%, decant carefully and replace with fresh 70% ethanol before proceeding.

7.2. Subsampling

The SWAMP standard requires identification of a minimum of 500 organisms per sample. A fixed-count random subsampling procedure is used (Caton, 1991):

1. Pour the entire sample through a 500 μm sieve. Rinse the jar into the sieve to recover all material.
2. Transfer the sieved material to the gridded Caton sorting tray. Distribute the material as evenly as possible across all grid cells.
3. Use a random number generator or random number table to select grid cells for sorting.
4. Under the dissecting microscope, remove all organisms from the selected cell using forceps, transferring them to a labeled petri dish or vial. Count each organism as it is picked.
5. Continue selecting and sorting random cells until the cumulative organism count reaches or exceeds 500. Complete the cell in progress even if the count exceeds 500 mid-cell.
6. Record the number of cells sorted, the total cells in the tray, and the total organism count.

Tip

Keep the residue (unsorted cells) in a labeled jar with 70% ethanol. The residue is needed for the re-sort QC check and may be required if additional identifications are needed later.

7.3. Identification

1. Separate sorted organisms into major taxonomic groups (e.g., Ephemeroptera, Plecoptera, Trichoptera, Diptera, Coleoptera, non-insect taxa) under the dissecting microscope.

2. Identify each organism to the lowest practical taxonomic level using standard keys (Merritt et al., 2019; Thorp & Rogers, 2015):
 - Insects: genus (family for early instars or damaged specimens)
 - Non-insect invertebrates: family or order (depending on group and specimen condition)
3. For specimens requiring higher magnification (e.g., chironomid head capsules, oligochaete chaetae), prepare slide mounts and examine under the compound microscope.
4. Record counts by taxon on the laboratory data sheet or directly into the data entry spreadsheet.

7.4. Voucher Specimens

1. Retain at least one representative specimen of each taxon as a voucher in a labeled scintillation vial with 70% ethanol.
2. Label each vial with: site ID, date, taxon name, identifier initials, identification date.
3. Store voucher vials in the BMI reference collection.

7.5. Data Entry and Metric Calculation

1. Enter identification data into the standardized spreadsheet or database.
2. Calculate summary metrics:
 - Total taxa richness
 - EPT (Ephemeroptera, Plecoptera, Trichoptera) richness
 - Percent tolerant taxa
 - Percent dominant taxon
 - Shannon diversity index (H')
3. If sufficient data are available, calculate the CSCI score using the CSCI R package or the online CSCI calculator (Mazor et al., 2016).

```
# Example: Shannon diversity from BMI count data
library(vegan)

# counts is a named vector of organism counts by taxon
counts <- c(Baetis = 120, Hydropsyche = 85, Chironomidae = 210,
            Simulium = 45, Optioservus = 30, Physa = 10)

H_prime <- diversity(counts, index = "shannon")
cat(sprintf("Shannon H': %.3f\n", H_prime))
cat(sprintf("Total taxa: %d\n", length(counts)))
cat(sprintf("Total organisms: %d\n", sum(counts)))
cat(sprintf("EPT richness: count EPT genera from full dataset\n"))
```

8. Quality Control

- **Sorting efficiency:** Re-sort 10% of processed samples (randomly selected). The re-sort technician examines all residue material from the original subsampling. Sorting efficiency must be greater than or equal to 90% (i.e., fewer than 10% of total organisms were missed).
- **Taxonomic verification:** Have a second identifier verify identifications for 10% of specimens. Agreement should be greater than or equal to 90% at the operational taxonomic level.
- **External QC:** For data submitted to SWAMP or CEDEN, submit 10% of samples to a SWAMP-certified taxonomic laboratory for independent re-identification.
- Record all QC results in the QC log.

9. Waste Disposal

Waste Type	Disposal Method
Ethanol from samples	Evaporate in fume hood or collect in flammable waste
Sorted residue (organic material)	Drain ethanol; dispose in standard trash
Used gloves and paper towels	Standard trash

10. References

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- Ode, P. R., Fetscher, A. E., & Busse, L. B. (2016). *Standard operating procedures for the collection of field data for bioassessments of california wadeable streams: Benthic macroinvertebrates, algae, and physical habitat*. California State Water Resources Control Board, Surface Water Ambient Monitoring Program. https://www.waterboards.ca.gov/water_issues/programs/swamp/bioassessment/sops.html
- Thorpe, J. H., & Rogers, D. C. (2015). *Ecology and general biology* (4th ed., Vol. 1). Academic Press. <https://doi.org/10.1016/C2010-0-65590-8>

11. Related SOPs

SOP	Title	Relationship
F610	Benthic Macroinvertebrate Sampling	Upstream: field collection
L002	Laboratory Safety	Required safety training
L601	Wet Mount for Microscopy	Slide preparation for ID

12. Training Record

Before sorting and identifying BMI independently, personnel must:

1. Read this SOP and [SOP-F610](#) in full
2. Complete [SOP-L002 Laboratory Safety](#) training
3. Review the major taxonomic keys (Merritt et al., 2019; Thorp & Rogers, 2015)
4. Complete a supervised sorting exercise achieving greater than 90% sorting efficiency
5. Correctly identify a training set of at least 30 taxa under supervision (greater than 85% agreement with reference identifications)
6. Be signed off by the PI or lab manager