

**SWAMP Bioassessment Procedures 2012**

# STANDARD OPERATING PROCEDURES FOR LABORATORY PROCESSING AND IDENTIFICATION OF BENTHIC MACROINVERTEBRATES IN CALIFORNIA

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Standard Operating Procedures for Laboratory Processing and Identification of Benthic Macroinvertebrates in California

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# LIST OF ACRONYMS & ABBREVIATIONS

| Acronyms & Abbreviations | Definitions  |
|--------------------------|--|
| AIS                      | Aquatic Invasive Species                                     |
| ABL                      | CDFG Aquatic Bioassessment Laboratory                        |
| BMI                      | Benthic Macroinvertebrates                                   |
| CDFG                     | California Department of Fish and Game                       |
| COC                      | Chain(s) of Custody  |
| DMT                      | SWAMP Data Management Team                                   |
| ID                       | identification   |
| KOH                      | potassium hydroxide  |
| LIMS                     | Laboratory Information Management System                     |
| mLs                      | milliliters  |
| MQO                      | Measurement Quality Objectives                               |
| NAWQA                    | USGS National Water Quality Assessment Program               |
| PPE                      | Personal Protection Equipment                                |
| QA                       | Quality Assurance  |
| QC                       | Quality Control  |
| SAFIT                    | Southwest Association of Freshwater Invertebrate Taxonomists |
| SOP                      | Standard Operating Procedure                                 |
| State Board              | California State Water Resources Control Board               |
| STE                      | Standard Taxonomic Effort                                    |
| SWAMP                    | Surface Water Ambient Monitoring Program                     |
| USGS                     | United States Geological Survey                              |



## INTRODUCTION

This document serves two purposes: 1) to provide requirements and recommendations to laboratories funded by the California State Water Resources Control Board's (State Board's) Surface Water Ambient Monitoring Program (SWAMP) or other laboratories producing SWAMP comparable data and 2) to document the standard operating procedure (SOP) for the SWAMP referee benthic macroinvertebrate laboratory, the Department of Fish and Game's (DFG) Aquatic Bioassessment Laboratory (ABL). This document is SWAMP SOP number 03 in the bioassessment series. It is intended for use with SWAMP's *Standard Operating Procedures for Collecting Benthic Macroinvertebrate Samples and Associated Physical and Chemical Data for Ambient Bioassessments in California* (Ode 2007). Instructions are provided for:

- Meeting SWAMP requirements for receiving SWAMP funding or producing SWAMP comparable BMI data;
- Reporting aquatic invasive species (AIS); and
- General taxonomic laboratory practices.

Additionally, the following ABL-specific procedures are described:

- Sample preparation for taxonomic identification, including cleaning, subsampling, and sorting;
- Enumeration and taxonomic identification of BMI specimens; and
- Internal quality control (QC) procedures and descriptive error reporting.

### A. HOW TO USE THIS DOCUMENT

This SOP is designed to serve two primary purposes within the SWAMP program. It describes and documents the processes used by the California Department of Fish and Game's (DFG) Aquatic Bioassessment Laboratory (ABL) in the role of referee laboratory for SWAMP. Additionally, this document provides guidance to non-SWAMP laboratories that are funded by SWAMP or wish to produce SWAMP comparable data. Policies set forth in this document are specified as either requirements or recommendations for laboratories producing SWAMP comparable data (Table 1). A descriptive summary of requirements, along with an overview of the SOP sections, follows Table 1.



**Table 1**

Procedural requirements and recommendations. The requirements listed must be met by laboratories receiving SWAMP funding, or wishing to produce SWAMP comparable data. Recommendations are suggested by SWAMP as means of producing quality data, but are not required.

| Relevant SOP Section(s) | Section                   | Element  | Description of Requirements  | Description of Recommendations  |
|-------------------------|---------------------------|--|--|---|
| 1.1                     | Laboratory Practices      | Aquatic Invasive Species (AIS) Policy                      | Report all invasive species to the Department of Fish and Game.  | None  |
| 1.2                     | Laboratory Practices      | Taxonomist Qualifications                                  | Taxonomists must be active members of SAFIT and follow SAFIT taxonomic training recommendations  | Minimum Bachelor's Degree in Entomology, Zoology or similar degree with relevant coursework in taxonomy |
| 1.3                     | Laboratory Practices      | Sorter/Picker Qualifications                               | None   | General entomology coursework   |
| 1.4                     | Laboratory Practices      | Reference Collection                                       | Laboratories must maintain an internal reference collection of vouchered specimens with confirmed IDs  | None  |
| 1.4                     | Laboratory Practices      | Reference Collection                                       | Staff must have access to up-to-date taxonomic information and dichotomous keys, and attend SAFIT workshops  | None  |
| 1.5                     | Laboratory Practices      | General Taxonomic Laboratory Practices                     | Maintain a lab SOP (can use this one as an example)  | None  |
| 1.6                     | Laboratory Practices      | Standard Taxonomic Effort                                  | Minimum taxonomic resolution must conform to the standard taxonomic effort levels specified by the project manager. SWAMP currently uses two standard levels defined by SAFIT (SAFIT Level I and SAFIT Level II) | None  |
| 2.1                     | Laboratory Sample Receipt | Sample Receipt   | Confirm that sample labels match chain of custody forms and all samples are accounted for. Confirm that any required ancillary sample information is present.  | None  |
| 2.2                     | Laboratory Sample Receipt | Sample Integrity Check                                     | Ensure all sample jars are intact, have no more than 50% (by volume) of sample material, and are filled to the top with 70% ethanol .  | None  |
| 2.3                     | Laboratory Sample Receipt | Hydrometer Preservation Check                              | Samples must contain a minimum of 70% ethanol. Check 10% of samples by project (or no fewer than one container per shipment). If failures are found, the entire batch must be checked.                           | None  |
| 2.4                     | Laboratory Sample Receipt | Sample Database Login and Laboratory Sample Identification | None   | Assign unique identifier to each sample to aid in sample tracking and data management.                  |





| Relevant SOP Section(s) | Section   | Element   | Description of Requirements   | Description of Recommendations  |
|-------------------------|---|---|---|---|
| 3.1                     | Sample Preparation                              | Sample Cleaning   | None  | Rinsing sample to remove detritus is highly recommended.  |
| 3.2                     | Sample Preparation                              | Sub-sampling  | Remove a random subsample of at least the target count of BMI from the surrounding matrix of detritus using a gridded tray or other random sub-sampling device. A minimum of 3 separate grids must be processed to ensure representativeness of the sub-sample. | None  |
| 3.2                     | Sample Preparation                              | Sorting   | None  | None  |
| 3.3                     | Sample Preparation                              | Remnant Jar Quality Control Check                             | Laboratories must have written SOP describing internal quality control check procedures. MQO for picking effectiveness is 90%.  | Perform internal laboratory QC check for picking effectiveness on at least 1 jar, or 10% (whichever is greater) of sample remnants per project. |
| 4.1                     | Identification and Enumeration of BMI Specimens | Determination and Enumeration of Non-Chironomidae             | Identify at least the target count of BMI to appropriate SAFIT STE Level. Taxonomists must indicate the developmental stage (larva, pupa, adult, or X for non-insect taxa) for each Final ID.   | None  |
| 4.1                     | Identification and Enumeration of BMI Specimens | Determination and Enumeration of Non-Chironomidae             | Any deviation from SAFIT taxonomic effort levels must be accompanied by one of SWAMP's explanatory codes (e.g., immature specimens, damaged specimens, etc.)  | None  |
| 4.1                     | Identification and Enumeration of BMI Specimens | Determination and Enumeration of Non-Chironomidae             | Taxonomists must store identify all taxa and voucher in separate vials.   | None  |
| 4.2                     | Identification and Enumeration of BMI Specimens | Clearing Specimens or Dissected Structures for Identification | None  | Perform as needed to properly identify specimens.   |
| 4.3                     | Identification and Enumeration of BMI Specimens | Determination and Enumeration of Chironomidae                 | Identify at least the target count of BMI to appropriate SAFIT STE Level. Taxonomists must indicate the developmental stage (larva, pupa, adult) for each Final ID.   | None  |
| 4.3                     | Identification and Enumeration of BMI Specimens | Determination and Enumeration of Chironomidae                 | Any deviation from SAFIT taxonomic effort levels must be accompanied by one of SWAMP's explanatory codes (e.g., immature specimens, damaged specimens, etc.)  | None  |



| Relevant SOP Section(s) | Section   | Element   | Description of Requirements  | Description of Recommendations  |
|-------------------------|---|---|--|---|
| 4.3                     | Identification and Enumeration of BMI Specimens | Determination and Enumeration of Chironomidae                   | Taxonomists must identify all taxa and voucher in separate vials or slides by taxa.  | None  |
| 4.4                     | Identification and Enumeration of BMI Specimens | Adding Qualified Specimens to a Laboratory Reference Collection | None   | Document reference collection holdings.   |
| 4.5                     | Identification and Enumeration of BMI Specimens | Sample Archiving  | Identified organisms must be archived for at least 5 years. There must be at least one vial per Final ID, and each vial must contain complete locality and determination labels.   | Locality labels should include unique sample tracking number if one exists.                               |
| 4.6                     | Identification and Enumeration of BMI Specimens | Minimum Storage Requirements                                    | Vials of identified organisms are stored for at least 5 years from sample date. Sorted sample residue is stored for at least 1 year from sample date. Unsorted sample remainder is stored for at least 2 years from sample date. | None  |
| 5.1                     | Internal Quality Control Check Process          | Quality Control Check Procedure                                 | Laboratories must have written SOP describing internal quality control check procedures.   | Perform quality control check on at least 1 sample, or 10% (whichever is greater) of samples per project. |

## B. PROCEDURE OVERVIEW AND REQUIREMENTS AND RECOMMENDATIONS FOR SWAMP FUNDED AND SWAMP COMPARABLE LABORATORIES

The procedures outlined in this document represent the methods used by the SWAMP referee laboratory, the Department of Fish and Game's Aquatic Bioassessment Laboratory (ABL). Non-ABL users of this document are not required to follow the ABL procedures described in this SOP; however, laboratories performing work funded by SWAMP and laboratories wishing to produce data that is SWAMP comparable should have written documentation of their laboratory procedures, and must meet the procedural requirements outlined in Table 1. Laboratories are free to adopt the ABL procedures detailed in this SOP, and incorporate these procedures into their own internal SOP as is relevant or helpful.

Table 1 outlines the requirements that laboratories must meet if they are SWAMP funded or wish to produce SWAMP comparable data. Table 1 also includes recommendations for these laboratories that are encouraged, but not required, by SWAMP. These recommendations provide information that SWAMP believes will assist laboratories in producing quality data.



## **SECTION 1: LABORATORY PRACTICES**

This section outlines ABL general laboratory practices and policies, including specifying aquatic invasive species (AIS) policy and reporting procedures, laboratory staff qualifications, information about reference collections, general taxonomy laboratory practices, and Standard Taxonomic Effort (STE). The following recommendations and requirements are made for SWAMP funded and SWAMP comparable laboratories:

### **Section 1.1, Aquatic Invasive Species (AIS) Policy**

It is required that all non-established AIS be reported to the Department of Fish and Game following the procedure outlined in this section.

### **Section 1.2, Taxonomist Qualifications**

It is required that taxonomists be active members of SAFIT and follow SAFIT taxonomist training recommendations. It is recommended that taxonomists hold a minimum of a Bachelor's Degree in Entomology, Zoology, or similar, with relevant coursework in taxonomy. It is desirable to have at least one staff member with post graduate training in one of the above mentioned fields.

### **Section 1.3, Sorter/Picker Qualifications**

There are no requirements, but general entomology coursework is recommended as desirable background for sorting and picking staff.

### **Section 1.4, Reference Collection**

It is required that laboratories maintain an internal reference collection of vouchered specimens with confirmed IDs. It is also required that staff have access to up-to-date taxonomic information and dichotomous keys.

### **Section 1.5, General Taxonomic Laboratory Practices**

Laboratories are required to maintain a laboratory SOP. Laboratories are welcome to use this document of ABL procedures as an example for their own SOP.

### **Section 1.6, Standard Taxonomic Effort**

The minimum taxonomic resolution used by laboratories is required to conform to the standard taxonomic effort (STE) levels specified by the project manager. SWAMP currently uses two standard levels of STE as defined by SAFIT (SAFIT Level I and SAFIT Level II).

## **SECTION 2: LABORATORY SAMPLE RECEIPT**

This section outlines ABL procedures for receiving samples, checking the integrity of samples upon receipt, verifying adequate preservation levels of samples, assigning samples unique identifiers, and logging the samples into the SWAMP database.



### **Section 2.1, Sample Receipt**

Laboratories are required to confirm that sample labels match chain of custody forms, must confirm that all samples are accounted for, and confirm that any required ancillary sample information is present.

### **Section 2.2, Sample Integrity Check**

Laboratories are required to ensure that all sample jars are intact, and have no more than 50% (by volume) of sample material, and that the jars are filled to the top with 70% ethanol.

### **Section 2.3, Hydrometer Preservation Check**

Laboratories are required to check that samples contain a minimum of 70% ethanol. Laboratories are required to check the preservation levels of 10% of samples by project per shipment (or no fewer than one container per shipment). If failures are found, the laboratory is required to check the preservation levels of all samples from the shipping batch.

### **Section 2.4, Sample Database Login and Laboratory Sample Identification**

It is recommended that laboratories assign a unique identifier to each sample to aid in sample tracking and data management.

## **SECTION 3: SAMPLE PREPARATION**

This section outlines ABL's procedures for preparing the samples for identification by the taxonomist. These procedures include sample cleaning to separate specimens from detritus, sub-sampling to remove a random selection of specimens for taxonomic identification, sorting specimens into taxa for taxonomic identification, and the procedure for the ABL internal quality control check of sample remnant.

### **Section 3.1, Sample Cleaning**

It is highly recommended that laboratories rinse the sample to remove detritus.

### **Section 3.2, Sub-sampling**

It is required that laboratories remove a random subsample of at least the target count of BMI from the surrounding matrix of detritus using a gridded tray or other random sub-sampling device. It is required that a minimum of 3 separate grids of the sample material be processed to ensure representativeness of the sub-sample.

### **Section 3.2, Sorting**

There are no specific requirements or recommendations for this procedure.

### **Section 3.3, Remnant Jar Quality Control Check**

Laboratories are required to have a written SOP describing internal quality control check procedures. The required MQO for picking effectiveness is 90%. It is recommended that laboratories perform an internal



laboratory quality control for picking effectiveness on at least 1 jar, or 10% (whichever is greater) of sample remnants per project.

## **SECTION 4: IDENTIFICATION AND ENUMERATION OF BMI SPECIMENS**

This section contains ABL's procedures for identifying and enumerating the prepared BMI specimens. This section also includes rules for handling specimen fragments, instructions for clearing specimens to aid in determinations, procedures for adding specimens to the ABL reference collection, and guidelines for sample archiving.

### **Section 4.1, Determination and Enumeration of Non-Chironomidae**

Laboratories are required to identify at least the target count of BMI to appropriate SAFIT STE Level. Taxonomists must indicate the developmental stage (larva, pupa, adults, or X for non-insect taxa) for each Final ID. Any deviation from SAFIT taxonomic effort levels must be accompanied by one of SWAMP's explanatory codes (e.g., immature specimens, damaged specimens, etc.). Additionally, taxonomists must voucher each taxon in a separate vial.

### **Section 4.2, Clearing Specimens or Dissected Structures for Identification**

It is recommended that laboratories perform clearing as needed to properly identify specimens.

### **Section 4.3, Determination and Enumeration of Chironomidae**

It is required that laboratories identify at least the target count of BMI to appropriate SAFIT STE level. Taxonomists must indicate the developmental stage (larva, pupa, adult) for each Final ID. Any deviation from SAFIT taxonomic effort levels must be accompanied by one of SWAMP's explanatory codes (e.g., immature specimens, damaged specimens, etc.). Additionally, taxonomists must voucher each taxon in a separate vial.

### **Section 4.4, Adding Qualified Specimens to a Laboratory Reference Collection**

There are no requirements for this section, but it is recommended that laboratories document reference collection holdings.

### **Section 4.5, Sample Archiving**

It is required that identified organisms be archived for at least 5 years. There must be at least one vial per final ID, and each vial must contain complete locality and determination labels. It is recommended that locality labels include the unique sample tracking number if one exists.

### **Section 4.6, Minimum Storage Requirements**

The following guidelines are required. Vials of identified organisms are stored for at least 5 years from sample date. Sorted sample residue is stored for at least 1 year from sample date. Unsorted sample remainder is stored for at least 2 years from sample date.



## **SECTION 5: INTERNAL QUALITY CONTROL CHECK PROCESS**

This section describes the ABL process for internal laboratory quality control checks, and the SWAMP minimum storage requirements for all of the sample components.

### **Section 5.1, Quality Control Check Procedure**

It is required that laboratories have a written SOP describing internal quality control check procedures. It is recommended that a quality control check is performed on at least 1 sample, or 10% (whichever is greater) of samples per project.



## SECTION 1

# LABORATORY PRACTICES

This section describes ABL policies on establishing and maintaining critical infrastructure for processing and identifying benthic macroinvertebrates. This laboratory infrastructure is critical to the production of high quality data. This section also describes the SWAMP procedure for reporting aquatic invasive species (AIS) that must be used by all SWAMP funded and SWAMP comparable laboratories.

ABL has several tools in place to facilitate the production of high quality data:

- Highly qualified taxonomists
- Access to up-to-date taxonomic literature and dichotomous keys
- A reference collection of verified and vouchered specimens
- Excellent general laboratory practices
- Infrastructure for sample tracking and data management

### 1.1 AQUATIC INVASIVE SPECIES (AIS) POLICY

SWAMP is committed to the reporting and control of aquatic invasive species (AIS). If an AIS is found by the BMI laboratory during any point of the laboratory process, it must be reported immediately to the Department of Fish and Game (DFG). DFG coordinates information sharing with the United States Geological Survey (USGS) invasives database, and with regional water quality boards as appropriate. This report must be emailed to [invasives@dfg.ca.gov](mailto:invasives@dfg.ca.gov), and must contain the following information:

Collection information:

- Name of person who collected sample
- Telephone number of collector
- Email address of collector
- Date collected
- Specific location of collection

Identification information:

- Species name
- Description
- Attach photographs (if possible)
- Name of identifying taxonomist
- Telephone number of identifying taxonomist
- Email address of identifying taxonomist
- Have you already reported this to USGS?



## 1.2 TAXONOMIST QUALIFICATIONS

Taxonomists are responsible for determining all specimen identifications. BMI taxonomists at ABL have the following minimum qualifications:

### Education

- Bachelor's degree or Master of Science (MS) in entomology, ecology, evolution, or related degree
- Coursework related to entomology, insect morphology, insect taxonomy, aquatic ecology, limnology, or insect ecology
- Graduate thesis or undergraduate research in taxonomy, phylogenetics, or ecology of benthic aquatic organisms

### Experience and Training

- Should have at least two years experience identifying BMIs
- Individuals new to a lab should train with more experienced taxonomy staff until the new staff member demonstrates an ability to produce datasets that meet internal QC standards
- Must be an active member of the Southwest Association of Freshwater Invertebrate Taxonomists (SAFIT); must regularly attend taxonomy training workshops offered by SAFIT

### Knowledge and Skills

- Must know how to access and use BMI taxonomic literature (contact SAFIT for assistance with identifying relevant taxonomic literature, if necessary)
- Must be proficient at using dichotomous keys to identify BMIs
- Must have experience in the use of light microscopy and standard specimen preparation techniques, including slide preparation, clearing, and dissection

## 1.3 SORTER QUALIFICATIONS

Sorters are laboratory technicians responsible for BMI sample cleaning and preparation for taxonomic identification and enumeration. Preparation steps include the subsampling and sorting of BMI for identification by taxonomists.

Qualified laboratory technicians should have a minimum of two years laboratory experience, with preference given to those who have experience processing bioassessment samples.

## 1.4 REFERENCE COLLECTION

ABL maintains a curated synoptic reference collection of BMIs. The reference collection provides an important resource for taxonomists when identifying BMI specimens. The collection should include the best available representative specimens of each BMI taxon that has been positively identified by laboratory taxonomists. The specimens should be organized in such a manner that they may be tracked and found at any time. Use extreme care when handling reference specimens to prevent damage. Criteria for reference collection additions are found in Section 4.4.





## 1.5 EXCELLENT GENERAL TAXONOMIC LABORATORY PRACTICES

Good laboratory practices exercised at ABL include, but are not limited to, the following:

- Maintain a written laboratory SOP that clearly documents all laboratory processes;
- Maintain sample tracking and data management systems including, but not limited to, data sheets;
- Clean microscopes, objective lenses, and light sources as necessary or as recommended by the manufacturer;
- Maintain easy access to all relevant scientific literature;
- Work on only one sample at a time;
- Do not mix taxa from different samples in the same Petri dish except for making side-by-side comparisons;
- When identifying a taxon for the first time, consider all lines of morphological and distributional evidence available;
- Use the reference collection and consult with other taxonomists in the laboratory as needed;
- Maintain communication and consultation with other BMI laboratories;
- Maintain good working conditions;
- Acquire and maintain minimal equipment requirements; and
- Have established protocols for workflow within laboratory.

## 1.6 STANDARD TAXONOMIC EFFORT

The Southwest Association of Freshwater Invertebrate Taxonomists (SAFIT) creates and maintains standard taxonomic effort (STE) lists of BMI that specify current taxonomic binomials and the taxonomic level to which specimens may be identified. Project managers are responsible for clearly communicating the STE level to the laboratory to ensure proper level of identification by taxonomists. Laboratories are responsible for following the current SAFIT STE.

SWAMP follows the nomenclature and taxonomic effort lists of SAFIT. The current SAFIT STE list may be found on SAFIT's website ([www.safit.org](http://www.safit.org)). Currently, SAFIT defines two different levels of STE.

- 1) Level I STE (SAFIT1) specifies genus-level identifications for all groups where possible, with the exceptions of monotypic species and Chironomidae which are identified to the family level.
- 2) Level II STE (SAFIT2) specifies species-level identification for all groups where possible, except for Chironomidae which are identified to genus or species group.



## SECTION 2

# LABORATORY SAMPLE RECEIPT

Before BMI samples can be prepared for enumeration and identification, the laboratory must receive the samples, check sample integrity and preservation, and assign each sample a unique sample identification code using the laboratory sample tracking system. As the designated SWAMP referee laboratory, ABL loads sample information directly into the SWAMP database.

### 2.1 SAMPLE RECEIPT

Confirm that the sample StationCode, SampleDate, CollectionMethod, Site ID and Replicate number matches between collection labels and chains of custody (COCs), and that all samples are accounted for, including all jars if a sample was too large to be preserved in one jar. Project managers may also send auxiliary sample information separately (e.g., templates for reporting data); verify that such data have been sent for all samples, if appropriate. Sample site IDs must be written legibly on labels using alcohol-proof instruments (e.g., pencil or India ink) and placed both inside and outside the sample jars. Most “permanent” marker inks are inappropriate because they are soluble in ethanol.

For all received samples, the following auxiliary data must be known. If data are missing, contact field crews or the project manager:

- Project name
- LabSample ID
- Station Code
- Sample Date
- Location Code
- Sample Replicate (if any)
- Jar number, and total number of jars
- Preservative and concentration
- Collection method (e.g., RWB, TRC, MCM)

Additionally, the following information should also be recorded on sample labels, COCs, and auxiliary information sent from the project manager:

- Site name
- Latitude, longitude, and site description



## 2.2 SAMPLE INTEGRITY CHECK

Upon sample receipt to the laboratory, confirm that each jar is no more than one-half filled with sample material. Any sample jar containing more than one-half sample material must be divided between two sample jars. Fill out two new locality labels, placing one inside the new jar, and sticking one to the outside of the new jar. The new jar is then filled with new 95% ethanol to a final concentration of at least 70% ethanol, filling the jar to within one inch of the top. Note any action taken on the COC, and in the notes section of the laboratory database sample log in. This sample split will also need to be entered into the database to ensure proper tracking throughout the laboratory processes.

If a jar is cracked or leaking upon receipt to the laboratory, transfer the affected sample to a new jar, label the new jar with sample information, and fill the jar with new 95% ethanol to within one inch of its top. Note any action taken on the COC, and in the notes section of the laboratory database sample log in.

## 2.3 HYDROMETER PRESERVATION CHECK

All sample jars should contain at least 70% ethanol solution to adequately preserve specimens, unless otherwise indicated by the project manager. To ensure integrity and prevent specimen decomposition during storage, a hydrometer should be used to check the preservative concentration of 10% of the project's samples per shipment. Additionally, the preservative concentration of samples must be checked if they contain:

- Mostly organic detritus;
- Algae; or
- Aquatic macrophytes.

These samples must be flagged during the sample log in procedure.

Materials Needed:

- Hydrometer (Figure 1)
- 150-mL graduated cylinder
- 95% ethanol
- Filtered funnel (Figure 2)
- Waste ethanol container

**Step 1.** Select at least 10% of the project's samples per shipment to be checked for adequate preservation levels.

**Step 2.** Add all samples with large amounts (at least 50% by volume) of organic detritus, algae, or aquatic macrophytes.

**Step 3.** Working with one sample jar at a time, decant approximately 100 mL of preservative into the graduated cylinder.





Figure 1: Hydrometer and 150 mL graduated cylinder used to check sample preservation levels.



Figure 2: Example of filtered funnel used to keep particulates out of the waste stream.

**Step 4.** Float the hydrometer in the graduated cylinder.

**Step 5.** Read the level of the meniscus on the scale of the hydrometer. Graduations on the hydrometer scale indicate percent alcohol content.

- If the preservation concentration is less than 70% ethanol:
  - o Carefully decant any remaining preservative from the sample jar through a filtered funnel into a waste ethanol container.
  - o Discard the preservative from the graduated cylinder through a filtered funnel into a waste ethanol container.
  - o Add fresh 95% ethanol to the sample jar to within one inch of the jar rim.
  - o During sample log in, record any actions taken on the COC and in the laboratory database system.
  - o Notify project manager and field crew about inadequate preservation.
- If the preservation level is equal to or greater than 70% ethanol:
  - o Return the ethanol to the sample jar from the graduated cylinder.
  - o Tightly cap the sample jar.

**Step 6.** Repeat this process from Step 3 for all remaining samples receiving a preservation check.

## 2.4 SAMPLE DATABASE LOGIN AND LABORATORY SAMPLE IDENTIFICATION

Samples are logged into a data tracking system. Other data tracking systems may include hard copy data sheets or a LIMS system. ABL uses the SWAMP Taxa Entry forms that link to the SWAMP database. Unique laboratory sample identification codes (LabSampleIDs) are assigned to each sample collection to assist in sample tracking and data management. The SWAMP Taxa Entry form assigns a unique laboratory sample identification code (LabSampleID) to each sample. This LabSampleID is the unique identifier for each sample and all of its components throughout all laboratory processes.

*Note: The California Department of Fish and Game's Aquatic Bioassessment Laboratory (ABL) is currently the only laboratory that loads its BMI data directly into the SWAMP database. If other laboratories process SWAMP-funded BMI work, a Microsoft Excel spreadsheet is populated with data that the SWAMP Data Management Team (DMT) loads into the SWAMP database. The spreadsheet and associated information may be found at: <http://swamp.mpsl.mlml.calstate.edu/resources-and-downloads/standard-operating-procedures#Taxonomy>. SWAMP database protocols specific to the ABL for entering lab sorting and taxonomy results are located in Appendix B.*



## SECTION 3

# SAMPLE PREPARATION AND PROCESSING

After BMI samples are received and logged in, samples are cleaned and prepared for taxonomic identification. The sample preparation process consists of removing BMI specimens from sampled benthic substrate detritus (i.e., cleaning), randomly selecting portions of the cleaned sample (i.e., subsampling), removing specimens from the subsample (i.e., picking), and assigning specimens to taxonomic lots prior to taxonomic identification (i.e., sorting). Figure 1 provides an overview of the sample preparation process.

Laboratory technicians, also called sorters, conduct all stages of sample preparation, and should have qualifications as specified in Section 1.2.

Raw samples are cleaned by separating BMIs from sample substrate and detritus. The cleaned sample is subsampled by distributing it to a gridded tray, then removing BMIs from randomly selected portions of the sample until the target count and three grids of the cleaned sample is selected for sorting. The subsampling process creates four components:

1. **Sorted sample vials** containing BMIs sorted by taxonomic order
2. **Unsorted sample remainder jar** containing cleaned sample not selected for sorting during the subsampling process
3. **Subsampled extras vial** containing any BMIs selected for sorting during the subsampling process that remain after the target count is reached
4. **Subsampled remnant jar** containing any material remaining after all BMIs are removed from the randomly selected subsample

### 3.1 SAMPLE CLEANING

During this process, fine sediment and large debris are removed from the sample yielding “cleaned” BMI specimens in a gridded subsampling tray.

Materials needed:

- Unsorted, raw sample from the field (including multiple jars, as relevant)
- Subsampling tray, such as 9 x 13 in. cake pan with 20 etched, 2 x 2.5 in. grids (*Note: ABL uses subsampling trays as above. If other tray is used (i.e., Caton tray), grid sizes will differ from those described below.*)
- 500- $\mu$ m standard testing sieve
- Ethanol catch container
- Ethanol waste container
- Sink with water supply and drain filter
- Funnel with filter, such as 4 in. funnel fitted with cone coffee filter (Figure 3)
- Forceps



- Step 1.** Open the sample jar(s) and pour all contents into the 500- $\mu$ m sieve over the ethanol catch container.
- Step 2.** Pour the ethanol from the catch container through the filtered funnel into the ethanol waste container to keep particles out of the waste container.
- Step 3.** Place the sieve containing the sample in the bottom of the sink. Gently spray the water over and through the sample in the sieve. As water washes through the sample, gently shake the sieve back and forth to dislodge fine sediment. Continue to gently shake the sieve under the water until the water runs clear and all fine sediment has been removed.
- Step 4.** Inspect large objects (e.g., rocks, sticks, leaves other large detritus) and remove any clinging BMIs using forceps. Rinse the large objects and detritus over the sieve after inspection and then discard them. Be vigilant for taxa that adhere tightly to substrates such as rocks and twigs to ensure that they have been removed before discarding the substrates.
- Step 5.** Transfer the rinsed sample from the sieve to the gridded sampling tray by firmly tapping the overturned sieve into the tray. With water, rinse any remaining sample in the sieve into the sample tray. Visually inspect the sieve and place any remaining BMIs into the gridded subsampling tray with forceps.
- Step 6.** Clean and rinse the sieve, sink, drain filter, and funnel filter, ensuring that no BMIs from this sample remain in the cleaning apparatus to contaminate the next sample processed.

### 3.2 SAMPLE PROCESSING: SUBSAMPLING, PICKING, AND SORTING

After the sample is cleaned, it is ready for subsampling. The sample is first subsampled to reduce sample size to the target count by removing BMI specimens from randomly selected portions of the cleaned sample. It is imperative that sample representativeness is maintained during this process. To ensure sample representativeness during subsampling, at least 3 grids must be used for subsampling, and approximately 100 BMI should be removed during each subsampling stage. BMIs are then picked from the subsample, and sorted into vials by taxon to be used for identification by taxonomists. The subsampling and identification processes may be performed by one person or separate individuals.

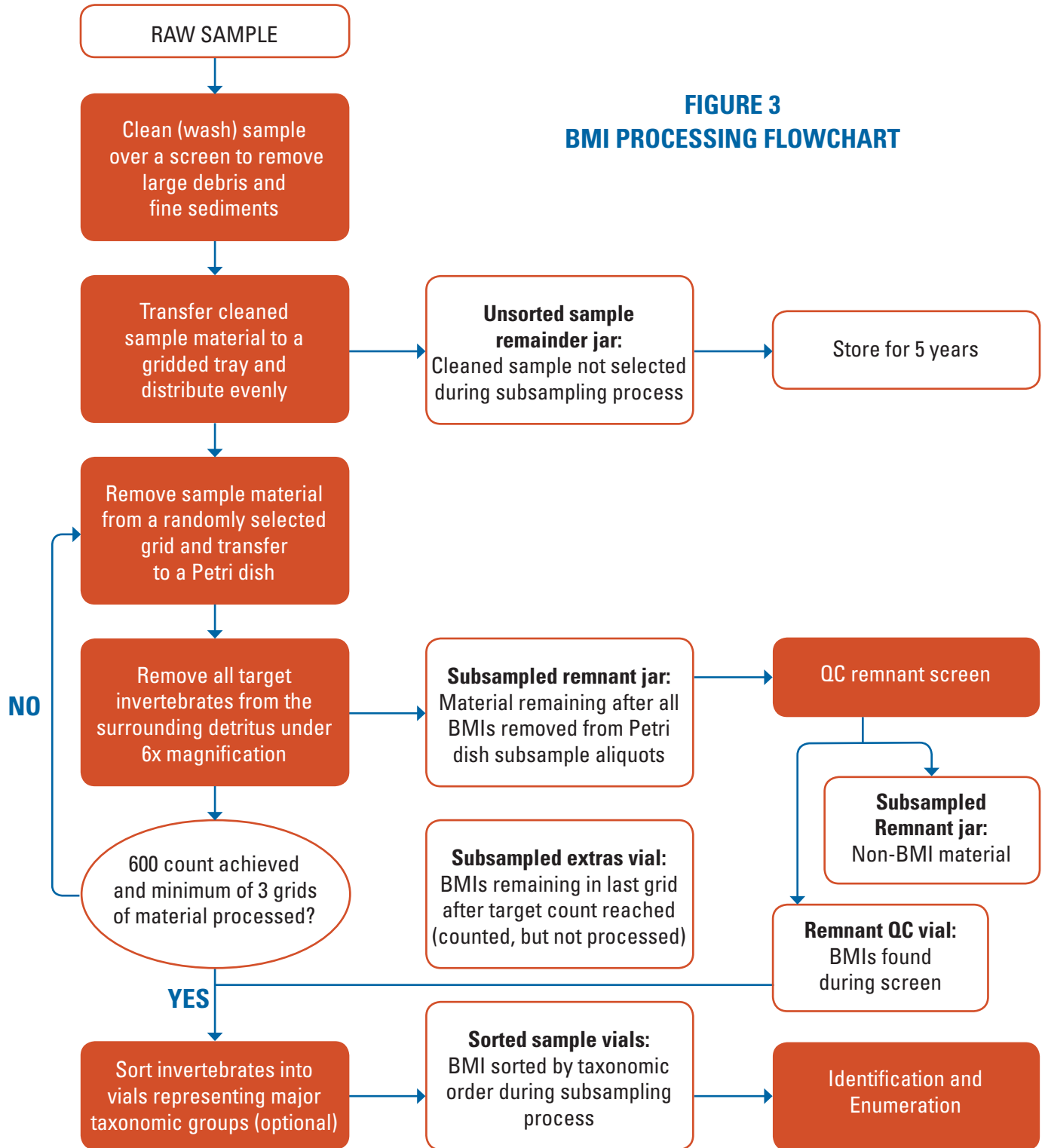
Two worksheets are utilized during sample processing, and may be found online at:

<http://swamp.mpsl.mlml.calstate.edu/resources-and-downloads/standard-operating-procedures#Taxonomy>:

- **Subsampling Worksheet:** used to record information about the grids selected for subsampling
- **Sorting Worksheet:** used to record information about how many individuals are sorted to each taxa.



**FIGURE 3  
BMI PROCESSING FLOWCHART**





The specimens are sorted into the following taxa:

- Ephemeroptera
- Plecoptera
- Odonata
- Trichoptera
- Hemiptera
- Coleoptera
- Diptera (other than Chironomidae)
- Chironomidae
- Oligochaeta
- Turbellaria
- Hirudinea
- Ostracoda
- Hydracarina
- Bivalvia
- Gastropoda
- Isopoda
- Amphipoda
- Decapoda
- Other

The sorted BMI specimens will then continue on to be identified and enumerated by taxonomists (Section 4). Products of the subsampling and sorting process are (Figure 3):

1. Sorted sample vials
2. Unsorted sample remainder jar
3. Subsampled extras vial
4. Subsampled remnant jar

Materials Needed:

- Gridded subsample tray (Appendix A) containing cleaned sample
- 1-dram snap-top vials for sorted BMI specimens (as many as necessary)
- Jar for unsorted sample remainder
- Jar for subsample remnant
- Random number generator or 20-sided die
- Petri dish
- Squirt bottle of 70% ethanol
- Dissecting scope
- *Subsampling Worksheet*
- *Sorting Worksheet*
- Forceps or scoopula
- Razor blade
- Multiple tally denominator

**Step 1.** Gently homogenize the sample by evenly spreading the sample onto the gridded subsampling tray. Sample thickness in the tray should be no greater than 0.5 in. If sample material exceeds a thickness of 0.5 in. in the first subsampling tray, it must be redistributed into two gridded subsampling trays to achieve a maximum thickness of 0.5 in. Target approximately 100 organisms per grid (2 x 2.5 in.).

**Step 2.** Write down the total number of grids that are covered with sample (maximum of 20 grids per subsampling tray) on the Subsampling Worksheet. Tray grids are arranged as five columns, four rows, and are numbered in rows from left to right, top to bottom. Randomly assign the grids a subsampling order using a random number generator or 20-sided die.

**Step 3.** Record the randomly determined subsampling order of grids on the Subsampling Worksheet.

**Step 4.** Divide the first selected grid into quarters with a razor blade, then further subdivide the quarters in half, creating a total of eight divisions within the selected grid. One-eighth grid (1.88 mm x 2.5 mm) is the smallest aliquot size that can be manipulated effectively.

**Step 5.** Begin by selecting the one-eighth grid subdivision in the upper left hand corner of the randomly selected etched grid. Organisms overlapping multiple grids or portions of grids are selected if the majority (i.e., > 50%) of the body is within the grid to be sorted.

**Step 6.** Using a scoopula or razor blade, transfer one-eighth of the selected grid to a Petri dish under a dissecting microscope. Scan the Petri dish under a dissecting scope at low power, and count the number of invertebrates from this first subsampling subdivision. Do not remove invertebrates for sorting at this point. The target count per aliquot is 100 organisms.

*Note 1: Steps 4 through 6 are critical to achieve a representative sample. If the estimated BMI count is greater than approximately 100 individuals, return the BMIs from the Petri dish to the gridded subsampling tray. Redistribute the unsorted, cleaned sample to evenly cover twice as many grids as were initially recorded in Step 2. Repeat Steps 2 through 6. If the aliquot target of 100 organisms is met, proceed to Step 7. If the aliquot target of 100 organisms is still not met, recombine the sample and redistribute evenly over double the original number of grids. If the estimated BMI count in one-eighth grid is fewer than 100 individuals, increase the subdivision size as appropriate to reach approximately 100 individuals per aliquot. Samples with very low BMI density may require processing of the entire sample to reach the project target BMI count of 600 specimens.*

**Step 7.** To prepare for picking, transfer the first selected aliquot into a Petri dish using the razor blade, forceps, or scoopula as necessary. Add 70% ethanol until specimens are completely covered. Gently agitate the Petri dish to evenly distribute the subsample within the dish. Place the Petri dish on a dissecting microscope stage, and adjust the magnification to a minimum of 10X.

**Step 8.** Scan the Petri dish for macroinvertebrates. Using forceps, remove the macroinvertebrates from the Petri dish and sort into vials according to taxonomic order. Enumerate the organisms for each order using a multiple tally denominator or other counter. This information will be entered into the *Sorting Worksheet* in Step 16.

**Step 9.** Scan the dish a minimum of three times, gently agitating the dish between each scan to expose any hidden organisms.

**Step 10.** After the dish has been scanned three times, and all macroinvertebrates have been removed, pour the remaining material from the Petri dish into the subsampled remnant jar, using ethanol to rinse as necessary.

**Step 11.** On the *Subsampling Worksheet*, record the total number of macroinvertebrates found during the subsample sorting process.

**Step 12.** Repeat the process from Step 5 until sorting is completed, moving through the list of randomly selected grids as recorded in Step 3. Sorting is completed when both of the following conditions are met:

1. The target count of organisms are subsampled from the gridded subsampling tray
2. Aliquots from at least three grids are sorted of the total sample volume is sorted

**Step 13.** If the target count is reached before you have finished scanning the dish, place all remaining macroinvertebrates into a new vial, labeled the subsampled extras, or “extras” vial.

**Step 14.** Record the total number of grids processed, the total volume of grids processed, grids volume analyzed, and total processing time on the *Subsampling Worksheet*.

**Step 15.** Transfer any remaining sample from the sample tray into the unsorted sample remainder jar, using ethanol to rinse the sample from the tray into the jar. Fill the sample remainder jar with ethanol to within one inch of the top of the jar.

**Step 16.** Create and populate locality labels for each jar and vial generated by the subsampling process. Locality labels must include the following information:

- State
- County
- Water body
- SWAMP station code
- Database tracking number
- Locality description
- Laboratory sample ID
- Collection date

Locality labels should be written in pencil or India ink.



Identical locality labels will be placed into each of the following containers:

- All sorted sample vials
- Unsorted sample remainder jar
- Subsampled remnant jar
- Empty remnant QC vial
- Subsampled extras vial (if applicable)

Products of subsampling receive labels as follows:

- **Sorted sample vials:** One locality label placed inside of each vial, taxon and number of individuals written on the back of each label
- **Unsorted sample remainder jar:** One locality label placed inside of the jar; one external sticky label with the following information: *project name, laboratory sample ID, and "unsorted sample remainder"*
- **Subsampled extras vial:** One locality label placed inside of the vial; "extras" written on the rear of the label
- **Subsampled remnant jar:** One locality label placed inside of the jar; one external sticky label that includes the project name, sample tracking number, and "remnant"
- **Remnant QC vial:** This vial will be used, if necessary, in the remnant QC process; one locality label created and bundled with empty remnant QC vial; "QC" written on the rear of the label

**Step 17.** Write the corresponding taxonomic information for each vial on the Sorting Worksheet. Include the following information:

- Project's target count
- Project's total count (fixed plus extras)
- Processing time (i.e., the amount of time from when the sample jar is first opened until all steps are completed, the original jar is stored, and the bundled sample vials and remnant jar are placed in the QC queue)
- Technician's name
- Sorting date

**Step 18.** Cap each sorted sample vial, and bundle them together with several rubber bands. Place the QC label for the empty remnant QC vial within the rubber bands on the outside of the bundle.

**Step 19.** Place the sorted sample vials, empty remnant QC vial, and subsampled extras vial into the taxonomic ID queue. The sample's remnant jar is placed into the remnant jar QC screen queue. Place the unsorted sample remainder jar into storage in accordance with the SWAMP two-year holding time requirement.



### 3.3 REMNANT JAR QUALITY CONTROL SCREEN AND MEASUREMENT QUALITY OBJECTIVE CHECK

As a QC measure, the remnant jar must be screened to ensure that all macroinvertebrates have been removed from the subsample aliquots during the subsampling process. A dedicated notebook should be established for remnant sample QC, where the following information should be noted:

- Date that the remnant sample is being screened
- Laboratory sample ID
- Name of the staff member (sorter) who initially processed the sample
- Name of the staff member performing the remnant sample QC screen
- The number of hours taken to initially process the sample
- The project name

After the remnant jar QC screen is complete, an error calculation is performed to assess sorting efficiency. This error rate is compared with the relevant MQOs, and corrective actions are taken if needed.

Materials Needed:

- Remnant jar for processing
- Small (9" x 6") white tray, such as Rubbermaid® JBI 2916
- Dissecting microscope
- Remnant QC notebook
- 70% ethanol squeeze bottle
- Forceps

**Step 1.** Carefully pour the remnant material into a small white tray. Using the ethanol squeeze bottle, rinse any remaining material left in the remnant jar into the tray.

**Step 2.** Move the tray from left to right under the microscope to screen for any remaining invertebrates.

**Step 3.** If any invertebrates are found, retrieve the remnant QC vial from the sorted sample vial bundle, fill it with 70% ethanol and add any invertebrates.

**Step 4.** Continue scanning the sample until it has been scanned for 10% of the original sample processing time, as noted in the remnant sample notebook. For example, if the original processing of the raw sample took two hours (i.e., 120 minutes), the remnant sample will be scanned for 12 minutes.

**Step 5.** After the remnant sample screen is complete, record the number of invertebrates found during the screen in the remnant screen notebook. Record the number of invertebrates on the back of the remnant QC vial label.



**Step 6.** Calculate the sorting efficiency MQO as in Equation 1 below. The frequency of sorting efficiency evaluation must be 100%, and the sorting efficiency must be equal to or greater than 90%. Record the results of this calculation on the *Subsampling Worksheet*.

Equation 1. Sorting efficiency calculation

$$\text{Sorting efficiency (\%)} = \left( \frac{\text{total number of organisms in initial sort}}{\text{total number of organisms after re-sort}} \right) \times 100$$

*Note: If the sorting efficiency is less than the MQO of 90%,*

1. Repeat the remnant QC screen from Step 2 to Step 6, until the MQO of 90% is achieved. In this instance, the first re-sort number becomes the initial sort value for the second calculation. For example, if the total number of organisms in the initial sort is 600, and the first re-sort finds an additional 100 organisms for a total of 700 organisms after re-sort, the sorting efficiency is (600 organisms/700 organisms) x 100 = 85.7% sorting efficiency. This is less than the sorting efficiency MQO of 90%, so the remnant must be screened again. An additional 3 organisms are found. To calculate the new sorting efficiency, the calculation is now (700 organisms/703 organisms) x 100 = 99.6% sorting efficiency.
2. Generate a Corrective Action Sheet as discussed in Section 5.

**Step 7.** Cap the QC vial and bundle it with the sorted sample vials. The non-BMI sample remnant can now be placed in the subsampled remnant jar and stored with the rest of the sample.

**Step 8.** Record the time taken to perform the remnant sample screen in the remnant sample notebook.



## SECTION 4

# IDENTIFICATION AND ENUMERATION OF BMI SAMPLES

Following cleaning, subsampling, picking, and sorting by taxa, the specimens in each sorted BMI vial (including any Remnant QC vials, as applicable) are identified and enumerated by taxonomists. The taxonomist enumerates and identifies non-Chironomidae taxa separately from Chironomidae specimens. Data are then recorded in the data management system. Forms and ABL data entry instructions may be found online at: <http://swamp.mpsl.mlml.calstate.edu/resources-and-downloads/standard-operating-procedures#Taxonomy>:

### 4.1 DETERMINATION AND ENUMERATION OF NON-CHIRONOMIDAE

Taxonomists work with one sorted vial at a time. During this process, every individual in the vial is identified to the project-specific, designated SAFIT STE level and is sorted by life stage (i.e., adult, pupae, larva/nymph, or undefined). Any deviation from designated SAFIT taxonomic effort levels must be accompanied by one of SWAMP's explanatory codes (e.g., immature specimens, damaged specimens, etc.). Taxonomists must voucher each taxon in a separate vial.

BMI specimens will occasionally become fragmented during the sample collection and sample cleaning processes. These fragments require special handling during the determination and enumeration process.

Rules for handling non-Chironomidae BMI fragments are as follows:

- Identify and enumerate all complete specimens (i.e., individuals with intact head, thorax, and abdomen).
- Do not identify and enumerate BMI fragments unless a head is present. Discard all headless fragments.
- Terminal abdominal segments must be present to identify insect pupae and adults to high taxonomic resolution.
- Head and thorax combinations from pupae and adult insects are only enumerated if at least some anterior abdominal segments are present. Do not attempt to match anterior fragments to the remainder of the body. Instead, discard these anterior fragments.

Specimens may be cleared using a 10% potassium hydroxide (KOH) solution as necessary to aid in identification, unless the project manager has indicated otherwise (Section 4.2). Following identification and life stage sorting, individuals in sorted vials are enumerated by taxa and life stage (e.g., five larval *Baetis*). These results are recorded in the data management system.



## Materials Needed:

- All BMI vials for one sample (including Remnant QC vials, as applicable), prepared and sorted to order
- Dissecting microscope
- Multiple tally denominator
- Squirt bottle of 70% ethanol
- Forceps
- Taxonomic keys
- Access to a reference collection
- Laptop with SWAMP Taxa Entry forms. *Note: Non-ABL laboratories may use other data management tools such as hard copy data sheets or LIMS.*
- 1-dram snap-top vials (or similar), as needed to separate specimens by life stage
- ¼-dram shell vials, or genitalia microvials, as needed for cleared, dissected, or fragmented specimens

**Step 1.** Transfer all of the specimens from one sorted vial into a Petri dish, adding 70% ethanol as necessary to cover the specimens.

**Step 2.** Move the Petri dish to the dissecting microscope stage.

**Step 3.** Identify familiar or common specimens to the designated SAFIT STE level, moving to one side of the Petri dish all specimens that cannot be identified with confidence by the taxonomist.

*Note 1: If the sample contains specimens that cannot be visually identified due to obscured identifying characteristics, the specimens may be cleared before continuing to Step 4. See Section 4.2 for clearing instructions.*

*Note 2: If the sample contains specimens that cannot be confidently identified upon sight by the taxonomist due to unfamiliarity, specimen rareness, or any other reason, specimens must be identified using an appropriate taxonomic dichotomous key. Contact SAFIT for a suggested resource list.*

**Step 4.** Sort identified insect specimens by life stage (i.e., adult, pupae, larva/nymph). For non-insect taxa, use the undefined life stage designation of “X”. These taxa are identified in the SAFIT STE.

**Step 5.** Enumerate all identified taxon specimens by life stage using a multiple tally denominator.

**Step 6.** Separate identified taxa by life stage into vials. Cover the specimens with 70% ethanol.





**Step 7.** Place determination labels inside of the vials. Determination labels should contain the following information:

- Taxon
- Life stage abbreviation (A, P, L, X)
- Specimen count
- Determining taxonomist name
- Year

Example: *Hesperoperla* sp. L 23  
det.: J. Slusark, 2007

**Step 8.** Place locality labels inside of the vials. Locality labels should contain the following information:

- State
- County
- Transect number
- Water body
- StationCode
- LocationCode
- Laboratory sample ID
- Replicate number
- Date of collection

Example: CA; San Diego Co., R1  
San Dieguito River (905DGSY1x)  
Santa Ysabel at Highway 79;  
ML# 19440 07/20/10

**Step 9.** Place specimen parts that are cleared or dissected during identification in a ¼-dram shell vial or genitalia microvial containing 70% ethanol and plug with cotton. Place the shell vial or microvial inside the primary taxon vial. Each shell vial should contain pieces from one individual specimen. Use as many shell vials as necessary.

**Step 10.** Record information from the current non-Chironomidae vial onto the bench sheet or the Taxa Entry form.

**Step 11.** Repeat the process from Step 1, working through all sorted non-Chironomidae vials in the sample one at a time until all vials have been identified and enumerated.

**Step 12.** After completing the identification and enumeration process of non-Chironomidae specimens, process sorted Chironomidae vial(s) as in Section 4.3.

## 4.2 CLEARING SPECIMENS OR DISSECTED STRUCTURES FOR IDENTIFICATION

Clearing is a chemical process that removes pigment from the specimen's exoskeleton in order to better see small or internal identifying characteristics. Clearing may be used for any project where taxonomic identification is the sole objective of BMI sampling; if other uses are intended (such as DNA or protein extraction), consult the project manager before clearing.

This process uses potassium hydroxide (KOH), a very strong base. Staff should wear personal protection equipment (PPE) as specified in the KOH Material Safety Data Sheet on file in the laboratory.

Materials Needed:

- 10% KOH solution
- 50-mL beaker
- PPE: safety glasses, nitrile gloves, lab coat
- Hot plate
- Forceps
- 70% ethanol
- 0.5 cc syringe
- KOH waste container

**Step 1.** Carefully place 2-3 KOH pellets into 50 mL of deionized water to create the clearing solution.

**Step 2.** Place the whole specimen or a dissected part of a specimen (e.g., abdomen from a caddisfly pupa or adult) into about 10 mL of 10% KOH solution in a 50-mL beaker.

**Step 3.** Warm the KOH solution gently on a hot plate for several minutes. Do not boil KOH solution. Clearing time will vary depending on the size of the specimen or structure being cleared (e.g., a small hydroptilid caddisfly may require two to three minutes; a large limnephilid caddisfly abdomen may take five to ten minutes to clear).

*Note: Alternatively, clearing can be performed overnight by placing a specimen in a sealed vial of 10% KOH at room temperature.*

**Step 4.** Check the specimen to determine if identifying characteristics are visible, or whether additional clearing time is required.

**Step 5.** Remove the specimen or structure from the KOH solution using forceps, and place it in a dish of 70% ethanol.

**Step 6.** Flush dissolved body tissues from dissected insect abdomens by inserting a 0.5 cc syringe filled with 70% ethanol. Inject ethanol into the open end of the abdomen to flush out dissolved tissues.



**Step 7.** When clearing is complete, return to Section 4.1, Step 4 to identify specimen.

### 4.3 IDENTIFICATION AND ENUMERATION OF CHIRONOMIDAE

Taxonomists work with one sorted Chironomidae vial at a time. Chironomidae specimens are sorted by subfamily (members of subfamily Chironominae are further sorted by tribe). Chironomidae subfamilies are as follows:

- **Chironomidae Subfamilies:**
  - o **Telmatogetoninae (marine)**
  - o **Podonominae (high elevation)**
  - o **Tanypodinae**
  - o **Buchonomyiinae (extremely rare)**
  - o **Diamesinae**
  - o **Orthoclaadiinae**
  - o **Chironominae**
    - **Tribe Chironomini**
    - **Tribe Tanytarsini**

Following sorting to subfamily, the sorted individuals are then identified using the method best suited to specimen size. Larger specimens are identified using a dissecting scope, while small Chironomidae are slide-mounted and identified using a compound microscope. Specimens may be cleared as necessary to aid in identification (Step 5, below). Following identification and life stage sorting, individuals in sorted vials are enumerated by taxa and life stage (e.g., five larval *Orthoclaadiinae*). Any deviation from SAFIT taxonomic effort levels must be accompanied by one of SWAMP's explanatory codes (e.g., immature specimens, damaged specimens, etc.). These results are entered directly into the data management system or SWAMP data loader.

Taxonomists must voucher each taxon in a separate vial.

BMI specimens will occasionally become fragmented during the sample collection and sample cleaning processes. Chironomidae fragments for all life stages are only identified if a head is present. Discard headless Chironomidae fragments.

Materials Needed:

- **Compound microscope**
- **Dissecting microscope**
- **Slides and coverslips**
- **Glycerin or 70% ethanol for wet mounting**
- **1-dram snap-top vials as needed**
- **CMC 10™(Masters Chemical Company, Inc)**
- **Slide dryer**
- **Squirt bottle of 70% ethanol**
- **Multiple tally denominator**



**Step 1.** Empty contents of the Chironomidae vial into the Petri dish. Add additional 70% ethanol as necessary to cover specimens in the dish.

**Step 2.** Place the Petri dish underneath the dissecting microscope.

**Step 3.** Identify and group larvae according to subfamily (or tribe for members of Chironominae).

**Step 4.** Place specimens sorted by subfamily or Chironominae tribe into snap-cap vials.

**Step 5.** Begin identifying specimens in each sorted vial, working through one vial at a time. Large specimens may be identified most easily using a dissecting scope, while smaller specimens may be identified most easily using a compound microscope.

- **Identification of large specimens:** Place the specimen in a Petri dish with 70% ethanol, and place the Petri dish under a dissecting microscope. Identify each specimen by sight or with a dichotomous key, as applicable. Place the identified specimen into the vial, cover it with ethanol, and insert the locality and determination labels into the vial. All specimens of a single taxon will be placed into one vial.
- **Identification of small specimens:** Wet mount the specimen onto the slide using either 70% ethanol or glycerin. Add a cover slip. Using a compound microscope at 100-1000X, identify each specimen by sight or with a dichotomous key, as applicable. Remove the identified specimen from the slide, and place it into a vial, cover it with 70% ethanol, and insert locality and determination labels into the vial.

*Note: Some Chironomidae specimens may require clearing to see traits required for identification. Taxonomists may either clear specimens as described in Section 4.2, or they may wet mount small specimens with CMC 10™ (Masters Chemical Company, Inc). Clearing small specimens with CMC 10™ requires slides to dry for at least 24 hours, and results in a permanent slide mount of the specimen.*

**Step 6.** Enter Chironomidae results into the data management system or SWAMP data loader.

#### 4.4 SELECTING SPECIMENS FOR LABORATORY REFERENCE COLLECTION

After all sample data have been recorded in the data management system or SWAMP database, the taxonomist may consider adding qualified specimens from the sample to the laboratory's reference collection. Specimens that may qualify for inclusion in the reference collection are:

1. Specimens of taxa previously unidentified by the laboratory, or otherwise rare.
2. Specimens that are exceptionally high quality examples of common taxa.
3. Specimens displaying unusual morphology or life stage not otherwise represented in the reference collection.



Select specimens that are mature, intact, and, when possible, are available in a series (several specimens of a taxon, or specimens representing a series of increasing maturity) from a particular sample. Specimens may be selected despite their condition if they represent the only or first verifiable record of a particular taxon. This step in sample processing is not mandatory and may be skipped if there are no qualifying specimens in the current sample. In this case, proceed to Sample Archiving, Section 4.5.

### Procedure for Adding a Qualified Specimen to a Reference Collection:

**Step 1.** Place the specimen in a 4-dram vial with a poly-seal screw cap or another, appropriately sized container. Cover the specimen with 70% ethanol.

**Step 2.** The candidate specimen is identified by a second taxonomist.

**Step 3.** If the second taxonomist confirms the identification of the candidate specimen, the second taxonomist populates a second determination label and places it into the specimen's vial.

*Note 1: If mounted specimens are selected, record the determination on the slide labels. The second taxonomist identifies the specimen as in Step 2 above, then adds initials on the slide labels to indicate agreement with the determination.*

*Note 2: If two taxonomists can not agree on the determination of a specimen, the laboratory should send the specimen to a qualified expert for identification. Contact SAFIT for assistance with locating a qualified expert.*

**Step 4.** If the new reference specimen was not in its own sorted sample vial, place a marker label in the original sorted sample vial of the removed reference specimen. The label should include the following information:

- Name of taxonomist removing specimen
- Designation of specimen
- Date of specimen removal
- "Added to reference collection"

## 4.5 SAMPLE ARCHIVING

Labeled vials containing identified Chironomidae specimens are stored in an empty cardboard vial box, along with the labeled, identified non-Chironomidae specimens. Slides are stored in a slide box that stays with the vial box containing the rest of the sample. The boxes containing specimens should be labeled with permanent marker on the outside of the box with project name, laboratory sample ID, and field sample date. Generate a *Vial Inventory Sheet* by printing identification and enumeration results, and include it in the vial box before moving the box to storage. This Vial Inventory Sheet serves as a record of specimens associated with the sample that may be located by laboratory staff at any time.

## 4.6 MINIMUM STORAGE REQUIREMENTS

All components of sample(s) are labeled and stored by the laboratory to allow for re-analysis as necessary. The sample components and required minimum storage times (measured from sample date) are as follows:

- Vials of identified organisms – at least 5 years
- Sorted sample residue – at least 1 year
- Unsorted sample remainder – at least 2 years

Samples stored in a temperature-controlled environment should be re-filled with 95% ethanol once a year as necessary while in storage. If samples are stored in an environment warmer than 80 F, they should be re-filled with 95% ethanol once per month while in storage.



## SECTION 5

### INTERNAL QUALITY CONTROL CHECK PROCESS

All laboratories funded by SWAMP or producing SWAMP comparable data must have a documented internal quality control process that includes routine re-identification of taxa. The following procedure is used at ABL.

After all samples from the project's sampling season have been identified and enumerated, and sample data have been entered into the laboratory database, the project's data are subject to an internal QC check. The internal QC check is performed by a QC taxonomist, a qualified taxonomist who did not perform the initial identification and enumeration. During the internal QC process, the QC taxonomist performs identification and enumeration on a randomly-selected 10% of the project's samples. The results of the 10% QC check are analyzed for error types and rates to determine whether the sample identification process has met SWAMP requirements. These analyses are currently performed by hand by the taxonomist using the Internal Quality Control Worksheet (available at: <http://swamp.mpsl.mlml.calstate.edu/resources-and-downloads/standard-operating-procedures#Taxonomy>). In the future, this process will be more automated with the use of the SWAMP QC Manager.

External quality control checks may also be performed by a designated referee laboratory, such as ABL, the SWAMP BMI referee laboratory. SWAMP is developing protocols to document its referee laboratory QC procedures.

#### SECTION 5.1 QUALITY CONTROL CHECK PROCEDURE

- Step 1.** Generate a spreadsheet of all BMI sample laboratory IDs from the completed field season of the project.
- Step 2.** Assign each sample a row number that will be referenced in the random selection process.
- Step 3.** Using a random number generator, randomly select 10% of the samples listed in Step 1 for internal QC check and error analysis.
- Step 4.** Retrieve all sorted vials and slides belonging to the randomly selected samples from the sample archive.
- Step 5.** Generate the *Internal Quality Control Worksheet*. Data from this worksheet are used for calculating error rates in Section 6.
- Step 6.** Verify the original count determination of all specimens for all vials and slides of the sample, following procedures in Section 4 of this SOP.



**Step 7.** In vials where QC determination confirms initial determination of all specimens in that vial:

- Return all specimens to the original sorted vials.
- Return the original locality and determination labels to each vial.
- Place a colored QC label in the vial, indicating that all specimens have been examined and no additional action is needed. The QC label contains the following information:
  - o “QC Checked”
  - o Determination by taxonomist name and year
  - o Laboratory name

Example:   QC Checked  
                  Det. J. Slusark 2011  
                  ABL Chico
- Record determination and count on the Internal Quality Control Worksheet.

**Step 8.** In vials where QC determination conflicts with the initial determination of all specimens:

- Return the specimen(s) to original, sorted vial.
- Return the original locality and determination labels to each vial.
- Create a new determination label with the corrected determination on it, and place it in the vial.
- Place a colored QC label in the vial, indicating that all specimens have been examined and no additional action is needed. The QC label contains the following information:
  - “QC Checked”
  - Determination by taxonomist name and year
  - Laboratory name

Example:       QC Checked  
                  Det. J. Slusark 2011  
                  ABL Chico
- Record the determination and count on the Internal Quality Control Worksheet.

**Step 9.** In vials where QC determination conflicts with the initial determination of some specimens in that vial: For specimens where QC determination confirms the initial determination:

- Return specimens to the original sorted vial.
- Return the original locality and determination labels to original sorted vial.
- Place a colored QC label in the vial, indicating that all specimens have been examined and no additional action is needed. The QC label contains the following information:
  - o “QC Checked”
  - o Determination by taxonomist name and year
  - o Laboratory name

Example       QC Checked  
                  Det. J. Slusark 2011  
                  ABL Chico





For specimens where the QC determination conflicts with the initial determination:

- Place these specimens with “corrected” determination into new vial(s) as appropriate, sorting by STE-determined taxa and life stage as in Section 4 of this SOP.
- Place a new determination label into the vial(s).
- Place the original locality label(s) into the vial(s).
- If the original locality label(s) is not available, place label(s) with the Laboratory Sample ID number into the vial(s).
- Place a colored QC label into vial(s), indicating that all specimens have been determined and no additional action is needed.
- Record the determination and count on the Internal Quality Control Worksheet.

**Step 10.** If a sorted sample vial could not be located for inclusion in the QC check, the laboratory must generate a *Corrective Action Report* in accordance with current SWAMP protocols. The QC taxonomist also records this event in the following ways:

- Enter specimens missing in the QC Determination data field, and record a zero in the QC count data field.
- Record missing vials on a vial inventory sheet that accompanies the sorted sample vials in storage.
- Immediately notify the Project Manager.

**Step 11.** Using the criteria listed in Section 4.4., determine if any specimens or vials are suitable for addition to the reference collection.

- Record the removal of specimens/vials from the rest of the sample on a vial inventory sheet.
- Add to the reference collection registry sheet and catalog appropriately



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# TERMS AND DEFINITIONS



## Terms & Definitions

| TERM                                 | DEFINITION   |
|--------------------------------------|--|
| Benthic Macroinvertebrate            | Bottom-dwelling invertebrates large enough to be seen with the unaided eye   |
| Cleaning                             | The preliminary washing step that removes some fine sediments and large detritus from sample   |
| Clearing                             | The process of removing tissue and pigment from BMI exoskeletons to aid in taxonomic identification  |
| Complete Specimen                    | BMIs with intact head, thorax, and abdomen   |
| <i>Measurement Quality Objective</i> | The performance or acceptance goals for the individual data quality indicators such as precision or bias   |
| Picking                              | The process of removing BMIs from a subsample  |
| Processing Time                      | The amount of time from when the sample jar is first opened until all steps are completed, the original jar is stored, and the bundled sample vials and remnant jar are placed in the QC queue |
| Sorting                              | The process of separating BMIs to genus for taxonomic identification   |
| Subsampling                          | The process of selecting a random subset of a sample for sorting   |
| Synoptic Collection                  | A collection of specimens representing important or major taxa found in a geographic region of study   |
| Taxon                                | (Plural: taxa) A taxonomic group of any rank, determined by the taxonomist to be a unit  |
| Target Count                         | The number of BMI that must be selected and identified per sample  |
| "Undefined" Life Stage               | The label assigned to non-insect taxa with non-descript life stages  |

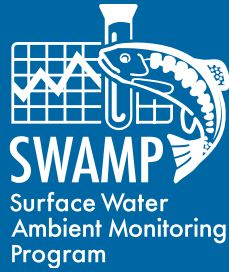


# APPENDIX A

## LABORATORY EQUIPMENT LIST AND SPECIFICATIONS

| Items  |   |
|--|---|
| Beaker 50-mL, Fisher Scientific                                  | Mason jar, ½ pt.  |
| CMC 10™(Masters Chemical Company, Inc)                           | Mason jar, 1 pt.  |
| Compound microscope 25x to 1000x magnification                   | Multiple tally denominator  |
| Dissecting scope 5x – 50x magnification                          | Petri dish, 100 x 15 mm polystyrene                                     |
| Ethanol catch container for sink – 1 liter Nalgene w/ cap        | PPE: safety glasses, nitrile gloves, lab coat                           |
| Ethanol catch container for sink – 4 liter reused EtOH container | Random number generator or 20-sided die                                 |
| Ethanol waste container – 55 gallon drum                         | Razor blade, one sided safety blade                                     |
| Fisher Standard 500-µm standard testing sieve                    | Shell vials (¼-dram), or genitalia microvials.                          |
| Forceps, Dumont Inox 5   | Sink with water supply and drain filter                                 |
| Funnel (4 inch diameter) fitted with coffee filter.              | Slide dryer   |
| Glycerin, 100% concentration                                     | Small (9" x 6") white tray, such as Rubbermaid® JBI 2916                |
| Graduated cylinder 150-mL  | Snap-top shell vials 1-dram   |
| Hot plate  | Squirt bottle for ethanol Nalgene cat. no. 2425-1002                    |
| Hydrometer, HB Instrument Company 50370                          | Subsampling tray, 9 x 13 in. cake pan with 20 etched, 2 x 2.5 in. grids |
| KOH solution 10%   | Syringe 0.5 cc  |
| KOH waste container, 50mL covered glass beaker                   | VWR micro cover glasses   |
| Laptop with SWAMP Taxa Entry forms.                              | VWR micro slides 25x75x1 mm   |





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