California's Surface Water Ambient Monitoring Program
SOP for Laboratory Processing and Identification of Benthic Macroinvertebrates in California

November 8, 2012

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CALIFORNIA DEPARTMENT OF FISH&GAME

Moss Landing Marine Laboratories

California Water Boards

SWAMP Surface Water Ambient Monitoring Program

Introducing the new SWAMP BMI Laboratory SOP!

- This SOP is the third in the SWAMP bioassessment SOP series.

- 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).

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Introducing the new SWAMP BMI Laboratory SOP!

- This is a different kind of SOP - it serves two purposes:
  1. Provides guidance to labs doing SWAMP work OR those wishing to be SWAMP-comparable
     - Specifies requirements that must be followed
     - Specifies recommendations that are suggested but not required
  2. Documents procedures of the SWAMP BMI referee lab, Department of Fish and Game's (DFG) - Aquatic Bioassessment Laboratory

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Introducing the new SWAMP BMI Laboratory SOP!

Instructions are provided for:

- Meeting requirements for receiving SWAMP funding
- Producing SWAMP-comparable BMI data
- Reporting aquatic invasive species (AIS)
- General taxonomic laboratory practices

Additionally, the following ABL procedures are described:

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

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Introducing the new SWAMP BMI Laboratory SOP!

- Today's focus will be on discussion of requirements and recommendations for laboratories doing BMI work under SWAMP contract, and other laboratories wishing to be SWAMP-comparable

- Consult SOP for specifics on ABL protocols. These protocols can be adopted for labs creating their SOP, as applicable.

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What is covered in the SWAMP BMI Laboratory SOP?

- Introduction - Overview of Requirements and Recommendations (Table 1)
- Laboratory Practices and Staff Qualifications
  - Aquatic Invasive Species reporting policy
- Laboratory Sample Receipt
- Sample Preparation and Processing
- Identification and Enumeration of BMI Samples
  - Laboratory Reference Collection
  - Sample Archiving
  - Minimum Storage Requirements
- Internal Quality Control Check Process

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Section 1: Laboratory Practices

- This section discusses:
  - Aquatic Invasive Species (AIS) policy and reporting
  - Laboratory staff qualifications
  - Reference collections
  - General laboratory practices
  - Standard Taxonomic Effort

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Section 1: Laboratory Practices

❖ 1.1 Aquatic Invasive Species 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.

New Zealand Mud Snails - Wikimedia

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Section 1: Laboratory Practices

❖ 1.1 Aquatic Invasive Species Policy

SWAMP is committed to the reporting and control of AIS. If an AIS is found by a BMI laboratory during sample processing it must report it to the DFG at **invasives@dfg.ca.gov**. The report 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

Has this already been reported to USGS?

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Section 1: Laboratory Practices

❖ 1.1 Aquatic Invasive Species Policy

- DFG Invasives Desk has volunteered to coordinate collection of AIS information
- DFG Invasives Desk will coordinate distribution of info to USGS invasives database
- Invasives Desk will contact regional boards that are affected by AIS finding
- DFG does collect data on non-BMI AIS; potential for coordination extends beyond BMI reporting

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Section 1: Laboratory Practices

❖ 1.2 Taxonomist Qualifications

- It is **required** that taxonomists be active members of SAFIT and follow SAFIT taxonomist training recommendations (www.safit.org).
- 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.

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Section 1: Laboratory Practices

❖ 1.3 Sorter/Picker 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.

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

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Section 1: Laboratory Practices

❖ 1.4 Reference Collections: ABL

- 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.

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Section 1: Laboratory Practices

❖ 1.4 Reference Collections

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. (Source list available at www.safit.org)

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Section 1: Laboratory Practices

❖ 1.5 General Taxonomic Laboratory Practices

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

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Section 1: Laboratory Practices

❖ 1.6 Standard Taxonomic Effort (STE)

SWAMP follows the nomenclature and taxonomic effort lists of SAFIT. The current SAFIT STE list is found on SAFIT's website (www.safit.org). Currently, SAFIT defines two different levels of STE.

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.

Level II STE (SAFIT2) specifies species-level identification for all groups where possible, except for Chironomidae which are identified to genus or species group.

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Section 1: Laboratory Practices

❖ 1.6 Standard Taxonomic Effort (STE)

The minimum taxonomic resolution used by laboratories is **required** to conform to the standard taxonomic effort (STE) levels specified by the project manager.

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Section 2: Laboratory Sample Receipt

❖ Introduction

This section discusses:

- receiving samples
- checking the integrity of samples upon receipt
- verifying adequate preservation levels of samples
- assigning samples unique identifiers
- logging the samples into the SWAMP database

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Section 2: Laboratory Sample Receipt

❖ 2.1 Laboratory 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.

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Section 2: Laboratory Sample Receipt

❖ 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.

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Section 2: Laboratory Sample Receipt

❖ 2.3 Hydrometer Preservation Check

- Check that sample preservation level is at least 70% ethanol
- Important to protect sample integrity, prevent degradation that would prohibit specimen identification
- ABL uses hydrometer to check preservation levels. Full ABL procedures available in the SOP

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Section 2: Laboratory Sample Receipt

❖ 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, and correct as necessary.

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Section 2: Laboratory Sample Receipt

- **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.

Labs doing SWAMP funded work can enter information into an Excel sheet that SWAMP DMT will use to upload into SWAMP database. Available at: http://swamp.mpsl.mlml.calstate.edu/resources-and-downloads/database-management-systems/swamp-25-database/templates-25

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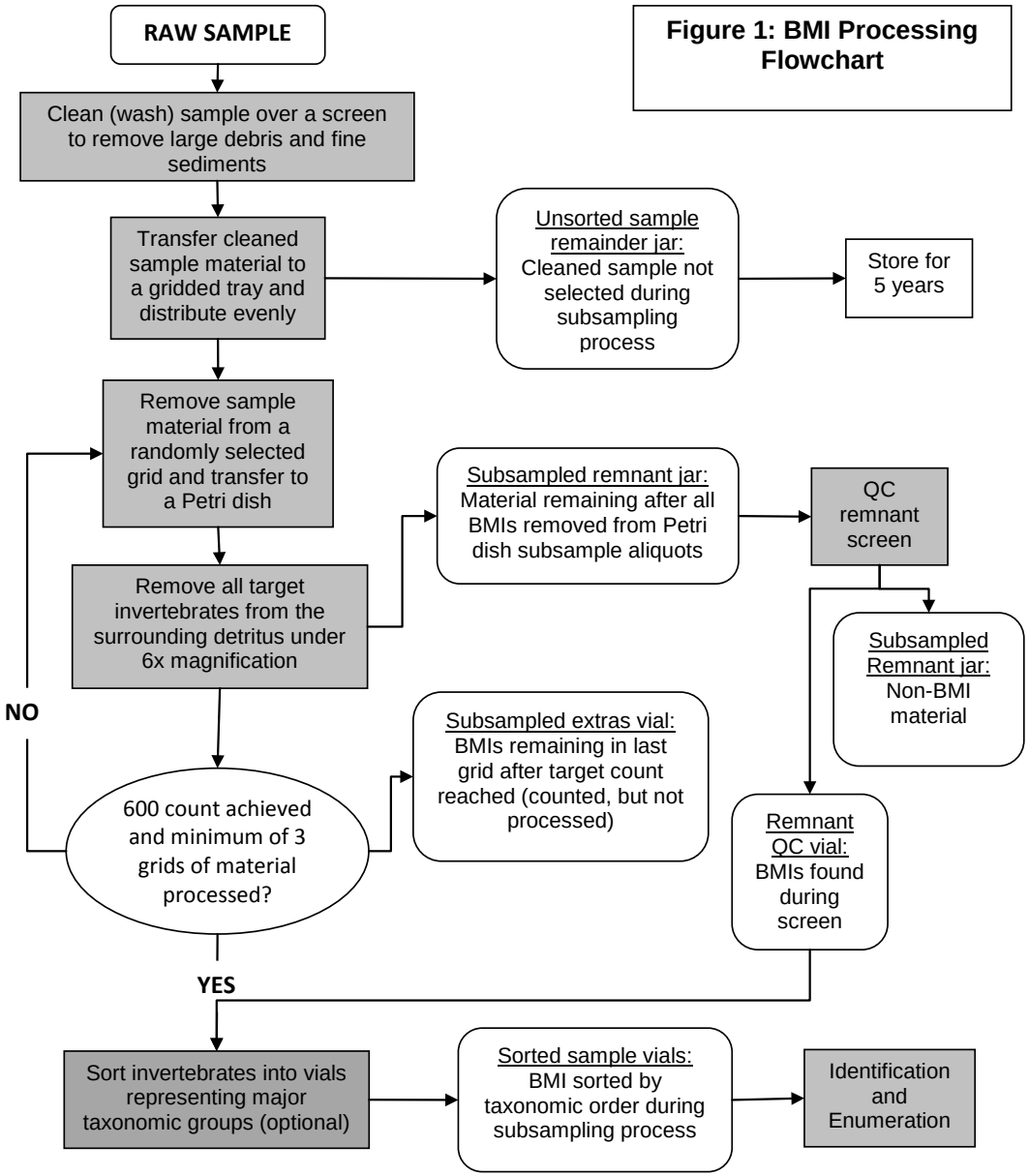
Overview of ABL Procedures



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Section 3: Sample Preparation

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Section 3: Sample Preparation

❖ Introduction

This section outlines ABL's procedures for:

- 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
- internal quality control check of sample remnant.

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Section 3: Sample Preparation

❖ 3.1 Sample Cleaning

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

- Full ABL procedure available in the SOP

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Section 3: Sample Preparation

❖ 3.2 Sub-sampling

The sample is subsampled to attain the target count of BMI specimens from randomly selected portions of the cleaned sample.

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Section 3: Sample Preparation

❖ 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.

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Section 3: Sample Preparation

❖ 3.2 Sub-sampling

It is imperative that sample representativeness is maintained during this process.

To ensure sample representativeness during subsampling, at least 3 grids must be subsampled and approximately 100 BMI should be removed from each subsampled portion. See SOP for details.

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Section 3: Sample Preparation

❖ 3.2 Sub-sampling

BMIs are then picked from the subsample, and sorted into vials by taxon for identification by taxonomists.

The subsampling and sorting (identification to order) processes may be performed by the same person or separate individuals.

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Section 3: Sample Preparation

❖ 3.3 Sorting

There are no specific recommendations or requirements for this procedure.

The ABL lab technicians sort (identify to order) as they remove specimens during the subsampling process.

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Section 3: Sample Preparation

❖ 3.3 Sorting

ABL sorts specimens into the following orders:

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

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Section 3: Sample Preparation

❖ 3.3 Remnant Jar Quality Control Check

- The remnant jar contains detritus that remains after BMI have been removed from the subsample and sorted

Laboratories are **required** to have a written SOP describing internal quality control check procedures. The picking effectiveness MQO 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.

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Section 4: Identification and Enumeration of BMI Specimens

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Section 4: Identification and Enumeration of BMI Specimens

❖ Introduction

This section contains ABL's procedures for:

- identifying and enumerating BMI specimens
  - Non-Chironomidae and Chironomidae in different sections due to handling related to size
- rules for handling specimen fragments
- instructions for clearing specimens
- adding specimens to the reference collection
- guidelines for sample archiving

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Section 4: Identification and Enumeration of BMI Specimens

❖ 4.1, 4.3 Identification and Enumeration of all BMI

- Labs 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.).
- Taxonomists **must** voucher each taxon in a separate vial.

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Section 4: Identification and Enumeration of BMI Specimens

❖ **4.2 Clearing Specimens or Dissected Structures for Identification**

What is clearing?

- 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.

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Section 4: Identification and Enumeration of BMI Specimens

- **4.2 Clearing Specimens or Dissected Structures for Identification**

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

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Section 4: Identification and Enumeration of BMI Specimens

❖ 4.3 Identification and Enumeration of Chironomidae

- ABL uses slightly different procedures for Chironomidae, based on size of specimens
- Same SWAMP requirements as for Section 4.1 (ID and enumeration of non-Chironomidae)

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Section 4: Identification and Enumeration of BMI Specimens

- **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.

ABL specific information available in the SOP.

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Section 4: Identification and Enumeration of BMI Specimens

❖ 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 a unique laboratory sample tracking number.

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Section 4: Identification and Enumeration of BMI Specimens

❖ 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.

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Section 4: Identification and Enumeration of BMI Specimens

❖ 4.6 Minimum Storage Requirements

Long term storage:

- Samples stored in a temperature-controlled environment should be refilled with 95% ethanol once a year
- Samples stored at 80 F or warmer should be refilled with 95% ethanol once a month

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Section 5: Internal Quality Control Check Process

The internal QC check is performed by a taxonomist who did not perform the initial identification and enumeration.

It is **required** that laboratories have an SOP describing internal quality control procedures.

It is **recommended** that a quality control check is performed on 10% of samples per project.

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Thank you!

- **Lilian Busse** (San Diego Regional Water Quality Control Board)
- **Will Hagan** (SWAMP QA Team)
- **Jim Harrington** (Department of Fish and Game Aquatic Bioassessment Laboratory)
- **Raphael Mazor** (South California Coastal Water Research Project)
- **Daniel Pickard** (Department of Fish and Game Aquatic Bioassessment Laboratory)
- **Doug Post** (Department of Fish and Game Aquatic Bioassessment Laboratory)
- **Andy Rehn** (Department of Fish and Game Aquatic Bioassessment Laboratory)
- **Brady Richards** (Department of Fish and Game Aquatic Bioassessment Laboratory)
- **John Sandberg** (Department of Fish and Game Aquatic Bioassessment Laboratory)
- **Marco Sigala** (SWAMP Data Management Team)
- **Beverly van Buuren** (SWAMP QA Team)
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SOP available online at:

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

Or Google search:

“SWAMP SOP”

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