

**LABORATORY STANDARD
OPERATING PROCEDURE FOR
BENTHIC MACROINVERTEBRATE
SAMPLE PROCESSING**

**BIOASSESSMENT
SERVICES**

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1.0 ORGANIZATIONAL STRUCTURE

BioAssessment Services (BAS) has been operated as a sole proprietorship by J. Thomas King since 1999 for conducting all phases of macroinvertebrate-based biological assessment. BAS is operated in Folsom, California where all samples are inventoried and stored. A conditioned room is dedicated for the storage of macroinvertebrate voucher collections and processing of samples. Mr. King manages all daily operations including client correspondence, management of subcontractor/s, sample inventory, macroinvertebrate identifications, and data entry and analysis. Mr. King also conducts benthic sampling and physical habitat assessments. Occasionally, BAS subcontracts to other taxonomists for identifications to meet project deadlines. All identification work contracted out is spot-checked prior to entering into a database. Quality of identification work performed by Mr. King is routinely evaluated by the California Department of Fish and Game's Aquatic Bioassessment Laboratory for taxonomic and enumeration accuracy, and conformance to standard taxonomic effort.

Subcontractors are routinely used for performing macroinvertebrate subsampling. Monica Murray is a primary subcontractor who has been providing subsampling services to BAS since 2002. Ms. Murray uses a Zeiss Stemi 2000-C stereomicroscope and Dolan Jenner fiber optic light source for performing all subsampling work.

2.0 BENTHIC SAMPLE INVENTORY

2.1 Background

2.1.1 Benthic samples collected in the field must go through standardized receiving and documentation procedures. A Chain of Custody (COC) form should be completed by the client prior to transfer of samples from the client's possession to BioAssessment Services (BAS). Sample location description labels should be provided in the container and on the exterior of the container. At the laboratory, each sample must be recorded in the Sample Log and given a unique sample ID. This ID is the BAS number, which provides unambiguous tracking of sample identity. In addition to logging of samples, adequate ethanol level is checked and ethanol added if insufficient. No biological stains are used by BAS.

2.2 Procedure

2.2.1 Place benthic samples on bench-top in ascending order according to site code. Client must be contacted if there are questions regarding sample identity. Client should be contacted if sites listed on the COC appear random unless the client indicated that the samples are to be processed "blind"; as opposed to being ordered from upstream to downstream or downstream to upstream.

2.2.2 The Sample Log is a three-ring binder, which is stored with the client binders. Open the Sample Log binder and locate the last BAS number recorded; binder labels group BAS numbers by calendar year. Start logging samples with the next BAS number after the last recorded entry. Record each distinct sample in the log including the BAS number, sample date, county, watershed, stream name, site code and transect number. If multiple containers were used to contain one sample, use the same BAS number for each container.

2.2.3 Check ethanol levels in the sample containers, and assure that benthic material in each container occupies no more than 70% of the volume of container. Most samples are transported directly by the client or BAS, but some samples may be shipped via third party carrier, subject to Department of Transportation regulations for shipment of hazardous materials. In the latter case, samples may arrive 'moist' without ethanol. In these cases, the client preserved samples with 95% ethanol, then, prior to shipment, decanted the ethanol and added just enough water to moisten benthic material prior to shipment. For samples that arrive 'moist', carefully decant excess water if necessary and fill container with 95 percent ethanol. Benthic samples should be split into two containers if benthic material occupies more than 70% of container volume. If sample emits odors of decomposition, check some benthic material under a stereomicroscope. Contact client if BMIs are not identifiable due to advanced decomposition.

- 2.2.4 Use the pre-printed BAS inventory number labels located in the Sample Log binder and attach to lid of container. Container lids may need to be cleaned prior to affixing labels. If lids are removed to add ethanol or confirm sample identity with internal label, make sure to keep lid with corresponding jar. Correct sample identity **MUST** be maintained and unambiguous- if there is any doubt regarding sample identity, cross-reference internal sample label with Sample Log and BAS number. Contact client if there is still a discrepancy.
- 2.2.5 If samples are to be subsampled by a subcontractor, place the inventoried samples in a box, and include the COC and copies of inventory log pages in a file folder. The inventoried samples are now ready for transfer to subsampling contractor.
- 2.2.6 During sample transfer to subcontractor, inform the subcontractor of timelines and special procedures. Sign and date the COC.

3.0 SUBSAMPLING PROCEDURE

3.1 Background

3.1.1 After samples are “logged in” (inventoried) and safely stored, the first task in processing the samples is to remove a subsample of benthic macroinvertebrates (BMI)s from each sample for later identification. The subsampling (often referred to as picking) should be performed as soon after the samples are collected as possible. The objective of subsampling is to randomly remove a fixed number of BMIs from a minimum of three discrete areas of the sample while maintaining the characteristics of the BMI assemblage of the whole sample.

3.1.2 The amount of time necessary to pick a sample is a function of multiple parameters including the number and types of BMIs, quantity and type of substrate/debris, and technician’s experience. Subsampling should be performed in a consistent and thorough, yet timely manner. The rate of subsampling can vary among technicians, and quality of work is emphasized over speed. The goal is to remove $\geq 90\%$ of the BMIs randomly from a minimum of three (preferably more) discrete portions of the sample. Quality of work is monitored with random checks of the remnant portion of the sample, noting the number and identity of BMIs missed. Technicians need to be aware of these QC checks, adjust their performance based on the results, and become proficient in recognizing the various groups of BMIs that are aquatic. No subsampling technician shall process benthic samples without thorough training and proven proficiency.

3.2 Equipment

- Number 35 sieve (0.5 mm mesh)
- Sink space, running tap water
- Subsampling tray with 20 grid pattern
 [approximate dimensions (cm): 32 x 23 x 4]
- # 5 forceps (Dumont non-ferrous recommended)
- Zoom stereomicroscope
- Glass Petri dish (9 cm wide)
- One-sided razor blades
- Spatula/scoop
- Shell vials (1 dram and larger)
- Vial holder (optional)
- 70% ethanol, and 30% water solution
- Location labels
- Wide mouth mason jars (4, 8, and 16 ounce)
- Self-adhesive labels, $\frac{1}{2} \times \frac{3}{4}$ in
- Fiber optic light source
- Calculator

- Tap water (in a wash bottle)
- Subsampling data sheet
- Hand tally counter
- Rubber bands (size 64)

3.3 Procedure

- 3.3.1 Revised procedures outlined in the CSBP in 2003 and the Surface Water Ambient Monitoring Procedure (SWAMP) specifies site composite samples. As a result, samples will most commonly be composited from an entire reach [reachwide benthic (RWB)] as specified in the SWAMP procedure. For larger river systems where a reach may consist of unwadeable deep water habitat, either SWAMP's targeted riffle procedure or the CSBP protocols may be used to collect benthic samples from the generally wadeable riffle habitat. The following procedure is based on a site composite and 500-organism subsample from the sample types listed above (RWB or targeted riffle) but be aware of other subsample sizes including a 600 organism subsample, which may become the standard subsample size for SWAMP projects.
- 3.3.2 Remove all project samples and copies of Sample Log sheets from the box and arrange the samples in ascending order by BAS number. Double check sample identity using the BAS number and site codes on jar with Sample Log information before processing samples.
- 3.3.3 For samples consisting of 0.5 liter or less of benthic material, transfer directly to number 35 sieve in the subsampling tray and add tap water to the tray/sieve to a depth of approximately one inch. Make sure the height of the sieve is greater than the height of the tray because you want rinse water to drain from the tray while the sample is retained in the sieve. Locate internal label in benthic material and check sample identification label with inventory record to assure sample integrity and assure that it cross-references with the BAS inventory number. Be aware that multiple jars may have been used to collect one sample. Additional jar/s will be labeled with the identical BAS number. Multiple jar contents must NOT be combined until their location information has been cross-referenced with the inventory log. If any irregularities are evident, STOP processing and contact Tom King. Rinse jar with tap water until entire sample (organisms, ethanol, detritus and substrate) is transferred to sieve. **Make sure the sample material that is greater than the sieve mesh size of 0.5 mm is always retained in the sieve during the rinsing process.** If total benthic volume exceeds 0.5 liter, transfer sample to large capacity bowl containing some tap water assuring that no sample spills out of the bowl. Gently add tap water to the bowl until the benthic material is submerged. Gently mix the benthic material in the bowl to assure the sample is homogeneous (composited). This step is necessary because field crews likely do not mix composite samples and they are likely stratified. After the sample is

- mixed in the bowl, transfer to sieve as described above.
- 3.3.4 Gently rinse the sample to remove fines and organisms less than 0.5 mm. It is best to fill the tray with tap water and gently agitate the sieve in the tray to remove fines and discard the rinsate (water in tray). Repeat washing process until rinsate is reasonably clear. Never allow water and sample to overflow from the top of the sieve; excess wash water must drain through the bottom of the sieve and out the tray, NOT from the top the sieve. Three rinse cycles are usually adequate but some large and/or sediment-laden samples may require additional rinses. Note that for samples with large benthic volume, the sample may need to be split into two or more portions for washing and then the rinsed sample portions should be combined in a large mixing bowl and gently mixed. After sample is mixed in bowl, gently transfer to sieve. Take precautions to work gently with the sample. Never manipulate a sample in the sieve without water; do not shake, rapidly stir, or grind the sample through the sieve. Do not use a direct stream from the faucet to wash the sample. Any harsh treatment can damage the BMIs, especially fragile worms and/or parts of insects such as abdominal gills, legs and cerci.
- 3.3.5 Inorganic material larger than 2 cm may be rinsed and removed from the sample. Remove green leaves, fresh twigs, and gravel, after rinsing thoroughly over the sieve; do not remove algae, skeletonized leaves or partially rotten wood, including small twigs. Lift the sieve evenly from the tray of water in a fashion that allows the detritus/sample to be evenly dispersed on the surface of the sieve. Watch for floating BMIs, which sometimes aggregate in a region of the sieve and clump together. If this occurs place the sieve (with the sample) back into the rinse water and re-distribute the sample/BMI (again, assure that the sample is confined by the sieve). As much as possible, attempt to evenly distribute the BMIs throughout the sample within the sieve.
- 3.3.6 After the sieve with sample has been removed from the tray, allow to drain excess water. Also drain the subsampling tray. Carefully invert the sieve with sample on to the subsampling tray. Gently tap the bottom of the sieve to dislodge detritus and BMIs from the sieve. To remove the remaining detritus and BMIs from the sieve, place the sieve at an angle under gentle flow of running tap water, and concentrate detritus/BMIs to one area of the edge of the sieve. Carefully use the spatula or ethanol washes to remove the remaining material and place into the tray. Finally, rinse any remaining sample in sieve to tray and inspect sieve for any remaining material.
- 3.3.7 After sample transfer from sieve to tray, back-flush the sieve and clean with brush to remove sand and material stuck in the sieve openings. Distribute the detritus and BMIs evenly over the tray using as many grids as necessary to obtain an approximate thickness of 0.5 inch or less when possible. Spread the detritus and BMI gently, and if necessary, move algae/detritus rather than cutting or pulling

- apart. The goal is to maintain BMI integrity and an even distribution of sample in the subsampling tray.
- 3.3.8 Complete the Laboratory Subsampling Worksheet including project name, BAS number and analyst's initials. To prevent labeling mistakes, cross-reference the sample label information with the BAS number and the Sample Inventory Log. Before starting to process the sample, record the number of grids covered by sample in the space provided (box at bottom of data entry area). Cross out the data entry area of grids without sample. For example, if the sample covers 16 grids, run a line through grid entry numbers 17-20. Using a random number chart, select three numbers between one and your highest grid number with sample. Record these as 1, 2, and 3, in their respective column. For example, if the first through third random numbers selected were 5, 9, and 2, then record a 1 under grid 5, a 2 under grid 9, and a 3 under grid 2. Do not erase mistakes; instead place a single line through the entry and record the correct entry adjacent to the error.
- 3.3.9 Starting with a subsample from the first random number grid, place half of the material from the first grid into a glass Petri dish for examination under the stereomicroscope (usually the grid is cut/separated diagonally with the spatula or razor blade). Add 70% ethanol to the subsample in the Petri dish until the subsample is covered. The quantity used is personal preference and serves multiple purposes. It preserves the sample, helps in manipulating the detritus while examining for BMIs, protects the BMIs from the abrasive action of picking, and serves as a solution to clean them before placing them into the specimen vial. Regularly check the material in the tray and dispense just enough 70% ethanol over sample in the tray to moisten it; then cover the bulk sample in the gridded tray with a tray cover. This will protect the sample from drying. Never allow the sample to dry.
- 3.3.10 In a methodological manner under the stereomicroscope, pick through the subsample, counting BMIs as you remove and place into a specimen vial ("500-count" vial). This vial, as well as the "extras" vial, must contain 70% ethanol. Place a label identifying each vial to the side. Use the hand tally counter to track the number placed into the 500-count vial. Never process more than one sample at a time and be sure to double check location information transfers.
- 3.3.11 Be aware of the invasive New Zealand mudsnail (*Potamopyrgus antipodarum*) that may be present in samples. For any snail with characteristics similar to the New Zealand mudsnail (NZMS), check the specimens from the sample with the archived NZMS you were provided. If there are similarities, contact Tom King as soon as possible.
- 3.3.12 Guidelines for selecting BMIs for the 500-count vial:

- a) Select intact BMIs with head, thorax, and at least half of an abdomen.
 - b) Spend extra time examining worms, especially small specimens (0.6 mm to 1 mm). In cases where small naidid worms are abundant, make a reasonable attempt to subsample most of them. Since many reproduce asexually through “budding”, worm fragments may have been viable prior to preservation. Excessive fragmentation during the picking process, however, can lead to their over-representation in samples as well.
 - c) Do not include zooplankton, surface dwellers, nematodes and organisms less than 0.5 mm in length (those that can easily pass through a 0.5 mm mesh).
 - d) Include pupal life stages.
 - e) Include the crustacean class Ostracoda (commonly referred to as “seed shrimp”).
 - f) Pick apart detritus such as leaves and twigs to locate BMIs that may be inside.
 - g) Determine that snails and clams were alive when preserved by checking for viscera in the shell. Discard empty shells.
 - h) Check all caddisfly cases for bodies. Transfer both case and body to specimen vial but discard empty cases.
- 3.3.13 Thoroughly check all material in the Petri dish for BMIs (adding more ethanol, stirring, and double checking the subsample after the initial examination is advised). The objective is to remove >90% of the BMIs from the portion subsampled. Transfer the processed detritus material (remnant), into an appropriated sized wide-mouth mason jar, covering with 70% ethanol. Place a remnant label into this jar. Remnant labels (location labels with the code REM written on the label) can be hand written as long as a minimum of the following is included: BAS number and site code. Place a self-adhesive sticker on the remnant jar lid with the BAS number and the code REM.
- 3.3.14 It is necessary to pick from a **minimum of three different areas (grids) of the tray** to minimize effects of possible heterogeneous distributions of organisms in the tray. When BMI abundance is low (i.e. the number of BMIs found in the first half grid is < 50), the second half of the first grid picked may be added to the Petri dish for examination. If the density is such that the number of BMIs found in the first half grid is > 35, pick a combination of half and whole grids so that a minimum of three different grid locations are processed. Occasionally, densities will be high enough that picking $\frac{1}{4}$ grids is warranted. After processing the first grid, enter whole, “W”, or half, “H”, (rarely “Q” for quarter) and record the number of BMIs placed into the 500-count vial to the subsampling worksheet.

- 3.3.15 Continue picking grids until 500 BMIs have been placed into the 500-count vial. At this time, finish the current grid, placing BMIs in excess of 500 in the “extras” vial. Continue to count these extra BMIs with the tally counter. Record the number of quarter, half, and whole grids processed, summing to get the total number of grids processed. For example, if 532 BMIs were subsampled from two whole grids and three half grids out of 16 grids with sample, the estimated total number of BMIs in the sample would be: $(532/3.5) \times 16 = 2432$. Note that this example includes 5 different locations of the tray. Record all variables used to calculate the estimated abundance value where indicated on the worksheet.
- 3.3.16 Products of subsampling includes two vials: the 500-count vial and the “extras” vial, each with a label including the BAS # inside. Additional vials may be needed when large organisms are present. Bundle the vials associated with one sample with a rubber band and place in the “completed” box. Store the remnant jar in the jar case with other remnants in numerical order. Place the unprocessed sample in a mason jar of the appropriate size (as small as possible to conserve space) and cover with 70% ethanol. Place the original label inside the jar with the unprocessed sample material. Place a self-adhesive label with the BAS number and the code “ORIG” on the lid of the jar. Store the jar in the case with the other original samples in numerical order.
- 3.3.17 In the event that the picker is unable to complete the processing of a sample in one day, the remaining sample in the tray must be well moistened with 70% ethanol and covered with the tray cover. A grid that has not been completely processed may be stored in a **covered** Petri dish overnight.
- 3.3.18 Clean the workstation. Insure that microscopes are clean and free of sand and dirt, then covered. Rinse Petri dishes and trays between samples with tap water. Rinse, dry, and store plastic sample jars for reuse.

4.0 MACROINVERTEBRATE IDENTIFICATION

4.1 Background

4.1.1 Benthic macroinvertebrates (BMI)s are identified to a standard taxonomic effort (STE) established by the Southwest Association of Freshwater Invertebrate Taxonomists (SAFIT). As of this SOP revision, SAFIT has established a level I STE, which specifies identifying most insects to the genus level and a level II, which specifies higher taxonomic resolution where possible and only in cases when peer-reviewed taxonomic keys are available. BioAssessment Services specializes in a level I STE but higher taxonomic resolution is often applied when requested with the exception of chironomids. Chironomids are either left at family (Chironomidae) or identified to subfamily/tribe. Deviations to the STE may occur for immature specimens and pupae.

4.1.2 Maintaining a minimum STE is crucial for comparing BMI data sets on temporal and spatial scales. It also provides a transparent framework for competitive bidding. Projects associated with FERC relicensing and urban stream monitoring have used a modified level I STE. The primary modification includes chironomid identification to subfamily/tribe. Currently, BAS routinely processes BMI samples at the level I STE and modified level I STE. BAS maintains keys for species level identification of most mayflies, groups of *Rhyacophila* and other taxa.

4.2 Equipment

- Stereomicroscope with zoom (magnification 5 to 50 X)
- Compound microscope
- Fiber-optic light source for stereomicroscope
- Forceps (#5 Dumont recommended)
- Teasing needles
- Petri dishes of various sizes
- Multiple tally denominator
- Taxonomic keys and references
- Current SAFIT list of taxonomic effort
- 70% ethanol solution
- 1 dram glass vials
- Microscope slides and cover slips
- CMP-9 low viscosity mounting medium for preparing slide mounts when
- Taxonomic labels

4.3 Procedure

4.3.1 Obtain subsampled organisms from subcontractor (if one was used) making sure

- all paperwork is completed and sample identities correspond to Sample Log. Prepare location labels using the following information: stream name, county, site code, transect #, date, BAS # and sampler initials if available. Use Times New Roman font size 7 for both location and taxonomic labels. For taxonomic labels include Order, Family and final identification. For monospecific taxa include the species name and always refer to SAFIT master taxa list for proper naming.
- 4.3.2 Print out a “BMI Identification Record” data sheet. Record BAS #, and date processed.
 - 4.3.3 Transfer the fixed subsample of organisms (usually 500 or 600) to a Petri dish using 70% ethanol solution. Do not process more than one sample at a time.
 - 4.3.4 BMIs are sorted and archived by taxon and transferred to individually labeled 1-dram vials containing the 70% ethanol. Each vial should contain from one to many individuals of one conclusively identified taxon. Include a location label in each vial. Make sure to rinse large BMIs in clean ethanol and inspect them for “tag-along” organisms.
 - 4.3.5 After processing each sample, tally the organisms corresponding to each taxon and record this number on the upper right corner of the taxonomic label and circle the number. Insert taxonomic and location labels in each vial, secure vial lid and tally the total organisms processed for the sample using the numbers written on the taxonomic labels. Record this number on the BMI Identification Record data sheet in “ID’er” field. Refer to this number during the data entry phase of processing. For a 500-organism subsample this number should be between 475 and 525 organisms ($500 \pm 5\%$). Contact the subsampler when deviations greater than $\pm 5\%$ of 500 occur in more than 5% of samples for any project sample set. The discrepancy between subsampler and taxonomist counts is possible and is due to many factors including enumeration error, loss of organisms during transfers, “tag-along” organisms, excessively damaged or decomposed organisms or non-benthic organisms.
 - 4.3.6 During processing of samples, immature or damaged taxa may require special treatment. Set aside any immature or damaged taxa that cannot be conclusively identified to the minimum STE but assign a tentative, “best guess” name (be sure to place a location label in the vial). Continue processing samples for the project and put aside any taxon that resembles the “best guess” immature or damaged specimen/s. The objective is to obtain a series of life stages from which secondary characters may be revealed or from which gradients of developing diagnostic characters may be used. Also, check the reference collection for similar taxa. If these efforts fail to provide conclusive identity of the immature or damaged taxon, then record it to the next higher taxonomic level (e.g. from genus to family). Another option is to attach the digital camera to the photo-tube on the stereomicroscope and send photomicrographs of the taxon to another taxonomist;

or mail the specimen to an interested taxonomist.

- 4.3.7 Record the estimated abundance by following instructions on BMI Identification Record data sheet. Record any special notes in the comments field of the BMI Identification Record data sheet. Record information such as organisms that were poorly preserved, excessively damaged, and taxa that couldn't be identified to the minimum STE.
- 4.3.8 After sample processing is completed for a project place the bundles of vials (one bundle of vials per sample) in an empty vial box and print out a 2x4 inch label showing the following:

VOUCHER COLLECTION
BENTHIC MACROINVERTEBRATES
PROJECT NAME – Season, Year

FRAGILE

(Contents: glass with 70% alcohol and 30% water)

BIOASSESSMENT SERVICES

5.0 DATA ENTRY AND MANAGEMENT

5.1 Background

5.1.1 Benthic macroinvertebrate (BMI) sample data are entered by project in an MS Access® relational database designed by BAS. The procedure includes multiple checks of data entry. After BMI data are entered, query templates are used to produce taxonomic lists, which are transferred to MS Excel® where metrics are calculated. Other queries may be run using the query templates. For SWAMP data, the SWAMP database Taxonomy Results Template is in the process of being evaluated for use. It is anticipated that data entry for SWAMP compatible data will be coordinated with the client. This SOP will be updated to reflect data entry requirements after SWAMP data entry modules have been tested and used.

5.2 Procedure

- 5.2.1 Turn on computer and open database: My Documents/database/active ver/BAS_2.6a updated MM.
- 5.2.2 Pull all boxes associated with the BMI voucher collection for the project to be entered. Remove first bundle of vials representing the first sample from the set and place on bench near computer.
- 5.2.3 Using the database switchboard, click on “Add/Edit Data”; then click “Sample Data”. The blue “Sample Main Form” will open. Scroll to bottom of page and click open new record (identified with an asterisk). Scroll up to top of page and fill in the fields: Sample ID (BAS number), Laboratory (BAS), Taxonomist (usually JTK).
- 5.2.4 Un-bundle the vials for the first sample and pull a vial. Record the taxonomic name in the “Final ID” field. This field is linked to a lookup table, which is linked to the Master Taxa Table. Enter the name until the taxonomic name is automatically revealed in the field. Assure that the name on the taxa label is identical to the name shown in the “Final ID” field and record the number of individuals in the “Quantity” field. Use tab to move to next field where you will repeat above process until all taxa and number of individuals associated with each taxon are entered for the sample.
- 5.2.5 If a taxon fails to appear in the Final ID field go to the Tables window of the database and select “tblMaster Taxa”. Search the “Final ID” records for the taxon in question (the records for the Final ID field are in ascending alphabetical order). If the taxon in question is absent from the list then go back to the Sample Main Form and select “Add New Taxa”. Make certain to select “add new record”. Then fill in the fields. The fields have lookup tables, which will automatically

show the Phylum, Class, and Order names as they are typed. You will need to fill in the genus and final id fields for the new taxa. Always refer to SAFIT's taxonomic list to record the Final ID field, and California Macroinvertebrate Laboratory Network (CAMLnet, January 2003 revision) for Tolerance Value and Functional Feeding Group designations. Check records for accuracy and click "refresh data". The new taxon is now entered into the Master Taxa table. However, you must close, and then open the Samples Main Form window for the new taxon to be added to the lookup table. After re-opening the Samples Main Form, select "Find" and enter the BAS number you were last working on. Then go to the Final ID field and enter the newly added taxon; make sure that it is activated by the lookup table.

- 5.2.6 After the data from the first sample are entered, go to the query window. Double click "bug count". Click query design button. Enter the range of BAS numbers for the project in the "criteria" field in the "Sample ID" column. For example: "Between 1700 and 1728". Click the exclamation point button to run the query. The query result will show the BAS number, number of organisms subsampled and identified and the number of taxa (which corresponds to the number of vials). Check these query results with the manual tally of organisms on the BMI Identification Record datasheet, and the number of vials associated with the sample currently on the bench-top. If there are no count discrepancies, proceed to the next sample. If there are discrepancies in the tally of organisms, and/or number of vials (taxa), then investigate the source of error and reconcile.

5.3 Product Queries

- 5.3.1 The product queries SOP is in development.

5.4 Data Management

- 5.4.1 Electronic data files including the database are stored in two folders: Active and Inactive. The active folder includes the database and client files accessed during the calendar year; the inactive files are client files, which have not been accessed during the calendar year. Active files are backed-up (copied) weekly to a 6 GB USB HDD. Inactive files are backed up once per year or after one of the inactive files has been accessed.
- 5.4.2 Hardcopy data sheets, COCs and other associated information on paper are stored in three-ring binders, each identified by client or project name. One binder is for the numerous small jobs (miscellaneous), which are partitioned by client with binder separators. All binders are stored in the sample repository room in the binder carousel.

6.0 QUALITY ASSURANCE

6.1 Background

6.1.1 Quality assurance procedures are embedded in the Standard Operating Procedures (SOP)s described in the previous sections. These include multiple checks and cross-referencing of sample identity throughout the entire process from sample inventory to identification of organisms and data entry. In addition, proficiency of subsampling contractors is evaluated prior to routine processing of samples. After a subsampling contractor has demonstrated subsampling proficiency, regular checks of sample “remnants” are conducted at a rate of approximately 10% from randomly selected samples. Remnants are the detritus and inorganic material remaining after organisms have been removed from samples. Inter-laboratory checks are also regularly conducted on voucher collections for assuring taxonomic accuracy, organism enumeration and conformance to standard taxonomic effort. The Department of Fish and Game’s Aquatic Bioassessment Laboratory is usually used for interlaboratory quality control.

6.2 Subsampling Quality

6.2.1 Remnant checks conducted during training are not included in the routine remnant checks used for quality control charts because all remnants are checked during the training process until proficiency is achieved. Proficiency is considered achieved after remnant recoveries of organisms are consistently less than 10% and there is no indication that the subsampling technician is missing appropriate benthic taxa. The learning process continues after training because samples from different aquatic habitat types may present new questions regarding the selection of applicable invertebrates to subsample. The subsampling contractor should seek regular feedback from the taxonomist and should be comfortable requesting guidance for handling potential problems and new situations.

6.2.2 As of this SOP revision date, 155 remnant samples have been evaluated as part of routine monitoring. Mean recovery of organisms for the 155 remnant samples was 5.0% and the standard deviation was 4.9%. A quality control chart is maintained, which tracks percent recovery and includes an upper warning limit (UWL) of 10%, which is approximately one standard deviation above the mean. The upper control limit (UCL) is 15% and is based on 2 standard deviations above mean recovery. Corrective action may not be implemented for occasional (5% of sample remnants) exceedances of the UWL, especially for samples with high filamentous algae content. Criteria for implementing corrective action include:

6.2.2.1 Exceedances of more than 5% of the samples of the UWL and any exceedance of the UCL.

- 6.2.2.2 Remnant recoveries of less than 2% occurring at a rate of more than 5% of the samples.
- 6.2.3 Corrective action for criterion 6.2.2.1 includes an examination of the sample matrix and dominant organisms from the sample that exceeded allowed recovery of organisms in remnant. Recovery exceedances are usually associated with samples containing high algal content and nauidid worms. Teasing nauidid worms from mats of algal filaments cause the fragile worms to fragment. Many of the nauidid worms could therefore be a product of subsampling artifact and so care must be taken when subsampling these samples. If algae-laden samples prove to be the primary factor contributing to exceedances, then no further action is taken as long as no systematic errors involving missing taxa were evident. If an exceedance is caused by factors not described above, then all samples are pulled from the project and their remnants checked. Organisms are pulled from any remnant exceeding the criterion listed in section 6.2.2.1. These pulled organisms are combined with the organisms originally subsampled, and 500 organisms are randomly selected, and metrics recalculated.
- 6.2.4 Corrective action for criterion 6.2.2.2 includes instructing the subsampling contactor to spend less time subsampling on a per sample basis. The goal is to approach the recovery mean with low standard deviation for maximizing subsampling precision and efficiency.

6.3 Identification and Enumeration Quality

- 6.3.1 A subset of processed samples is regularly evaluated by independent taxonomists, usually through the Department of Fish and Game's Aquatic Bioassessment Laboratory, Chico Office. Samples associated with county stormwater projects and Federal Energy Regulatory Commission projects are regularly checked for conformance to minimum standard taxonomic effort, enumeration of organisms and taxonomic accuracy. Clients managing small projects (e.g. 12 samples or less) with limited budgets usually do not request external quality evaluation of voucher collections. Unless client specifically requests that the voucher collections be returned to them, voucher collections are stored by BAS for up to four years after data/report submittal to client. After four years specimens may be discarded and vials recycled. Spot checks of voucher collections stored for four years or more indicate that the current storage process is satisfactory: location and taxonomic labels are clearly legible and BMI specimens are in good condition, although some loss of pigmentation is evident.
- 6.3.2 As of the revision date of this SOP, there has not been a consistent process for external quality evaluations of voucher collections. Optimally, the client requesting the work should select samples and contract with an independent taxonomist. However, due to limited time and experience, clients usually request that BAS handle the contracting, sample selection, and delivery of samples to an

- outside laboratory. For most projects, randomly select 10% of the samples for external quality evaluation. Occasionally, for urban stream systems, the most taxonomically rich sample can be submitted to DFG to maximize the cost-benefit of the validation. Some urban stream samples contain few (<10) taxa but the cost of the external validation is the same regardless of the number of taxa.
- 6.3.3 As of this SOP revision, results of external quality evaluations have rarely resulted in corrective action and all results have indicated that taxonomy was performed in accordance with standard taxonomic effort and quality was very good. However, minor problems have been identified by the quality control taxonomists. Minor adjustments to processing have been initiated to reduce the incidence of “tag-along” (small organisms hidden within gill filaments of larger organisms for example) as described in section 4.3.5.
- 6.3.4 There are many factors contributing to the decision to initiate corrective action. While the taxonomist must use good judgment when deciding whether or not to initiate corrective action, the client should also be consulted after review of the quality control report. There are unambiguous cases, which must result in corrective action. One case is systematic error: Any taxon consistently misidentified. Another case is not identifying the organisms to the minimum standard taxonomic effort, except for very immature specimens or pupae. Other cases become more difficult to assess and should be discussed with the client. Factors to consider include threshold at which enumeration errors have meaningful effects on results, and threshold at which incidental taxonomic errors have meaningful effects on results.
- 6.3.5 As described above, corrective action must be initiated for cases of potential systematic error. Corrective action can best be described with an example. If the original taxonomist was not able to recognize the diagnostic characters separating the water mite genera *Testudocarus* and *Torrenticola*, then the former would be combined with the latter, which would be identified by the QC taxonomist as a systematic taxonomic error. Corrective action for the example described above would include re-examining all project vials containing *Torrenticola*. Corrections of systematic error include updating the database, spreadsheet files and metric calculations. Using the example above, an incidental taxonomic error would occur if the original taxonomist knew the taxonomic differences between the two mite genera and correctly identified them in other samples but simply made a mistake by placing a *Testudocarus* individual in a vial containing *Torrenticola* specimens. This mistake should be corrected but it is probably unnecessary to re-examine all specimen vials for the project containing *Torrenticola* and *Testudocarus* individuals.