PROCEDURES FOR LABORATORY ANALYSIS OF SEDIMENT SAMPLES FROM MARINE AND ESTUARINE WATERS FOR BENTHIC INFAUNAL COMMUNITY ASSESSMENT

FOR THE RWQCB 8 SWAMP PROGRAM

(Part of Appendix G)

Southern California Bight
1998 Regional Marine Monitoring Survey
(Bight'98)

Field Operations Manual

AND

Quality Assurance Manual

As prepared by:
Southern California Coastal Waters Research Project (SCCWRP)
Westminster, CA

LABORATORY ANALYSIS PROCEDURES FOR SEDIMENT SAMPLES COLLECTED FROM MARINE AND ESTUARINE WATERS FOR BENTHIC INFAUNAL COMMUNITY ASSESSMENT

INTRODUCTION

This document describes laboratory procedures to be followed in the analysis of SWAMP sediment samples collected from marine and estuarine waters of RWQCB 8 for benthic infaunal community assessment purposes, following the procedures used in the collection and analysis of the same types of samples for the Southern California Bight 1998 Regional Marine Monitoring Survey (Bight'98).

The procedures described are based upon existing practices utilized in POTW monitoring programs within the region and those employed during the 1994 Southern California Bight Pilot Project (SCBPP). Some modifications have been made to assure data comparability and to facilitate the coordination of the quality control steps required for the Bight'98 infaunal survey. It is the responsibility of each participating laboratory's supervisor to assure 1) these procedures are followed during sample processing and analysis, 2) all quality control steps are implemented, and 3) copies of all records, forms, and documents generated in the process are securely maintained on file until all aspects of the survey and resulting reports are completed.

In overview, the process of sample analysis consists of four steps after receipt of the sample in the laboratory; 1) the sample is washed and transferred to preservative, 2) All organisms are removed from the debris contained in the sample and sorted into major taxa groupings, 3) the biomass is estimated for these major taxa groupings, and 4) all specimens in the sample are identified and enumerated. Quality control activities are required for the steps 2 and 4. These include repeating the procedures at each of these steps for a sub-set of samples. Results of this process are used to determine whether the measurement quality objectives (MQOs) established for each of these steps are met.
In addition, taxonomists must participate in a series of workshops jointly sponsored by Bight'98 and the Southern California Association of Marine Invertebrate Taxonomists (SCAMIT) which will focus on taxonomic problems arising during analysis of the Bight'98 samples. These workshops culminate in a synoptic review of the data set compiled from all participating laboratories.

Copies of this manual are available on the web site of the Southern California Coastal Water Research Project at http://www.sccwrp.org/

1. SAMPLE TREATMENT AND STORAGE

1.1 Upon receipt in the laboratory, samples will be in formalin fixative and must be washed and transferred to preservative. The removal of formalin is necessary for two reasons. Formaldehyde becomes increasingly acidic over time and prolonged exposure damages organisms with calcareous structures (e.g., shelled mollusks). Also, formaldehyde is a noxious, potentially dangerous chemical; its replacement with ethanol makes subsequent handling of the sample safer. Other benefits of the washing process are the removal of excess silt from mudballs that may have broken down during fixation and,

LABORATORY ANALYSIS PROCEDURES FOR SEDIMENT SAMPLES COLLECTED FROM MARINE AND ESTUARINE WATERS FOR BENTHIC INFAUNAL COMMUNITY ASSESSMENT

in some cases, the opportunity to separate the bulk of organisms in a sample from the inorganic debris through the application of an elutriation process.

1.2 The samples are to remain in buffered fixative for at least 72 hours. No sample should remain in fixative for longer than two weeks.

1.3 The preservative to be used for infaunal samples is a 70% solution of ethanol. It is recommended that the preservative be buffered with marble chips, especially if the ethanol used is produced by industrial distillation rather than fermentation.

1.4 Procedure

1.4.1 Working under a fume hood and with eye protection, decant fixative through a 0.5mm or finer mesh sieve.

1.4.2 After decanting the formalin, refill the sample container with water, agitate gently by swirling, and wash the entire sample into the sieve.

1.4.3 Gently wash the sample with a low-pressure stream of water to remove any fine silt.

1.4.4 Using a spatula and wash bottle containing preservative, transfer the sample back to the sample container, top the sample with preservative, and tightly affix the lid.

1.4.5 Place an internal label in each sample container bearing the station name, sampling date, split number (if more than one container is used. Labels are to be written in pencil or indelible ink on 100% rag-paper, poly-paper, or other paper suitable for permanent wet labels.

1.4.6 After each sample is washed, closely examine the sieve to assure that all organisms have been removed to avoid cross contamination of subsequent samples.

1.4.7 Elutriation. If a sample is primarily coarse sand, subsequent sorting can be greatly facilitated if inorganic material in the sample is separated from the lighter organic debris and organisms by the following elutriation process.

1.4.8 After washing the formalin from the sample, spread the sample material out in a shallow pan and cover with water.

1.4.9 Gently agitate the sample by hand to allow the lighter fraction of debris and organisms to separate from the heavier material.

LABORATORY ANALYSIS PROCEDURES FOR SEDIMENT SAMPLES COLLECTED FROM MARINE AND ESTUARINE WATERS FOR BENTHIC INFAUNAL COMMUNITY ASSESSMENT

1.4.10 Decant the water off with the lighter material through the sieve. Repeat the process several times until no more material is observed being carried off in the decanted water.

1.4.11 Collect the material carried off in the decanted water into a small sample container, top with preservative, and return to the original sample container along with the balance of the sample material. Fill the container with preservative and tightly affix the lid. Be sure that both the containers are properly labeled with internal labels.

1.5 Store infaunal samples in a safe and secure manner protected from environmental extremes. Avoid temperatures above 30°C as high temperatures will lead to evaporative loss of preservative.

1.6 Routinely inspect all samples to assure that the container closure is tight and the preservative level adequate. If evaporative loss of preservative is evident, top-off the sample using 100% ethanol. The use of 70% ethanol for this purpose will lead to dilution of the sample preservative because of the different evaporation rates of ethanol and water.

2. SAMPLE SORTING

2.1 Sorting is the process by which organisms (that were alive at time of collection) in a benthic sample are removed from the organic and inorganic residues that compose the sample and sorted into broad taxonomic categories for subsequent taxonomic analysis. Sorting must be accurate and complete to assure the value of all the subsequent steps in the sample analysis process.

2.2 Procedure

2.2.1 All laboratories participating in the Bight'98 infaunal survey have established sorting procedures that are compatible with the aims of this survey. The following points stipulate those elements essential to the process or unique to the Bight'98.

2.2.2 Begin the sorting process by filling out a Bight'98 Sorting Record form with the sample name, date, sorter's name, and date sorting begins. If the sample consists of more than a single jar, they are to be treated together as a single station. Make sure you have all jars composing the sample.

2.2.3 Sort the sample under a stereo microscope. It is recommended that the sample be sorted in small volume increments.

2.2.4 The entire sample is to be sorted. If an unusual sample is encountered for which sorting of an aliquot may be a reasonable alternative, the laboratory

LABORATORY ANALYSIS PROCEDURES FOR SEDIMENT SAMPLES COLLECTED FROM MARINE AND ESTUARINE WATERS FOR BENTHIC INFAUNAL COMMUNITY ASSESSMENT

supervisor is to contact the Bight'98 Benthic Specialist. The decision whether to allow sorting by aliquot will be made by the Benthic Specialist.

2.2.5 All sorting must be done in 70% ethanol, with care taken to assure that the sample being sorted is always fully covered with alcohol.

2.2.6 The organisms removed from the sample are sorted into the lots for which biomass will be estimated. These are:

Annelida	Mollusca	Misc. Echinodermata
Arthropoda	Ophiuroidea	Other Phyla Other Phyla is a single collective lot containing all other phyla.

2.2.7 Remove all individual organisms (including nematodes) and fragments from the sample with the exception of foraminiferans and planktonic species or life stages. All fragments, such as decapod chelae and legs, should be placed in their respective taxa lots. Sorters are to be instructed "If in doubt, pick it out".

2.2.8 Note on the Sorting Record form the number of taxa lots composing the sorted sample, the number of containers used if sample is split, and the time (to the nearest ½ hour) required to sort the sample.

2.2.9 Aggregate the taxa lots into one or more sample containers. Each taxa lot should be internally labeled with the station name (a four digit number). Place an internal label in each sample container bearing the station name, sampling date, split number (if more than one container is used). Labels are to be written in pencil or indelible ink on 100% rag-paper, poly-paper, or other paper suitable for permanent wet labels.

3. BIOMASS ESTIMATION

3.1 An estimation of biomass is determined, based upon wet-weights of the six taxonomic categories into which the organisms were sorted. Biomass is reported to the nearest 0.1 gram (wet weight).

3.2 Procedure

3.2.1 All laboratories participating in the Bight'98 survey have established wet-weight biomass procedures that are compatible with the aims of this survey. The following points are intended to stipulate those elements essential to the process or unique to the Bight 98 survey. Either of the two methods used by participating laboratories for removing excess preservative prior to weighing may be used: draining organisms on a fine sieve, followed by air-drying for a measured 5 minutes on absorbent paper; or pouring the sample

LABORATORY ANALYSIS PROCEDURES FOR SEDIMENT SAMPLES COLLECTED FROM MARINE AND ESTUARINE WATERS FOR BENTHIC INFAUNAL COMMUNITY ASSESSMENT

into a funnel fitted with a fenestrated plate, followed by the application of gentle vacuum to pass air through the sample until liquid is no longer visible in the funnel stem. Because biomass is being estimated as wet weight, both techniques are considered to yield equivalent results.

3.2.2 Biomass estimations are required for each of the six taxa lot created in the sorting process. These are:

Annelida	Mollusca	Misc. Echinodermata
Arthropoda	Ophiuroidea	<>

3.2.3 All taxa lots should be inspected by a taxonomist prior to weighing to assure that all individuals and fragments have been properly grouped and that foraminifera and plankton have not been included in the sample. The mollusk lot should also be inspected to assure that empty mollusk shells are not included in the biomass estimation.

3.2.4 An electronic balance capable of reading to 0.01 gram is to be used for biomass estimation. The balance must be calibrated prior to conducting the analysis.

3.2.5 Begin the biomass estimation process by filling out the Bight'98 Biomass Estimation Record with the sample log number, station, date, technician's name, and date of biomass estimation. If more than one container comprises the sample, make sure you have all containers for the sample.

3.2.6 Remove hermit crabs from shells prior to weighing.

3.2.7 To avoid biasing the biomass data, very large organisms are to be weighed separately. For example, the chance capture of a megafaunal animal such as an Allocentrotus fragilis, would typically result in an echinoderm biomass tens or hundreds of times that contributed by all other echinoderms in the sample. In this case, separate biomass estimations are to be determined for the Allocentrotus and for the remaining specimens comprising the taxa lot. If a technician is uncertain whether an organisms should be treated in this manner, the laboratory supervisor should be consulted.

3.2.8 The measured net biomass is to be recorded to the nearest 0.01 gram (wet weight). Record the report biomass of each taxa lot (and any large individuals) to the nearest 0.1 gram (wet weight) on the Biomass Estimation Record. The gross, tare and net weights of each measurement must be recorded.

LABORATORY ANALYSIS PROCEDURES FOR SEDIMENT SAMPLES COLLECTED FROM MARINE AND ESTUARINE WATERS FOR BENTHIC INFAUNAL COMMUNITY ASSESSMENT
(Page 7 of 17)

4. TAXONOMIC ANALYSIS AND ENUMERATION

4.1 The object of taxonomic analysis is to accurately identify all organisms contained within each sample to the lowest possible taxonomic category and to provide an accurate count of the organisms in each identified taxon.

4.2 The goal of the Bight'98 infaunal survey is to provide species level identifications whenever possible. However, because of difficulties in the taxonomy and the lack of expertise within the participating laboratories the following exceptions are made:

- Nematodes are identified to phylum Nematoda
- Kinorhynchs are identified to phylum Kinorhyncha
- Oligochaete annelids are identified to class Oligochaeta
- Hirudinean annelids are identified to class Hirudinea
- Podocopid ostracods are identified to order Podocopida
- Harpacticoid copepods are identified to order Harpacticoida

4.3 The number of organisms reported must account for all organisms in a sample alive at the time of collection. Care must be taken to avoid reporting empty mollusk shells or crustacean molts in the data. Fragments of bilaterally symmetrical organisms will be identified and counted only if the fragment includes the anterior end of the organism. For radially symmetrical organisms (e.g., ophiuroids, anthozoans) only fragments bearing the majority of the oral disk will be identified and counted.

4.4 Epibiotic (fouling) organisms are noted as present but not quantified. These data are not included in the final survey data. The level to which epibiotic organisms are identified is left to the discretion of each laboratory.

4.5 Parasites are noted as present but not quantified. Ectoparasites of fish such as Livoneca, which may be temporary members of the benthic community, are counted.

4.6 Each participating laboratory will use their own taxonomy bench sheets for recording the identifications and counts.

4.7 Nomenclature and orthography follows that used in the Edition 3 of the Southern California Association of Marine Invertebrate Taxonomists' taxonomic listing (SCAMIT 1998). This list represents a consensus for standard usage of taxa names in POTW monitoring programs in the Southern California Bight.

4.8 Taxonomists are to employ two standard notations (Voucher and Exclude) for the annotation of their data sheets While other non-standard notation may also be used, the use of these standard notations is required where applicable. In addition, the Exclude

LABORATORY ANALYSIS PROCEDURES FOR SEDIMENT SAMPLES COLLECTED FROM MARINE AND ESTUARINE WATERS FOR BENTHIC INFAUNAL COMMUNITY ASSESSMENT

code will be included as part of the electronic data record. See the Bight'98 Information Management Plan for the proper form for this field for data submission.

4.9 Voucher Notation

4.9.1 Form: The annotation employed for this purpose is the letter V followed by the number of specimens removed from the sample (i.e., V-3)

4.9.2 Purpose: To note the removal of specimens from a sample for use as vouchers. Use of this notation is essential to the process of quality control and assessment. Removal of organisms without annotation confuses the resolution of discrepancies during quality control re-analysis, and leads to overstatement of error rates.

4.9.3 Rule of Use: Removal of any specimens from a sample to the voucher collection is clearly noted on the bench sheet by means of the Voucher notation..

4.10 Exclude Notation

4.10.1 Form: The letters EX written on the row of the bench sheet containing the data record for the taxon to be excluded

4.10.2 Purpose: Provides an aid to data analysis when calculating metrics using the number of taxa present (e.g., diversity, species richness). This field in the final data set represents the taxonomist's recommendation that the reported taxon be excluded from counts of the number of taxa reported in the sample.

4.10.3 Rule of Use: The Exclude annotation is made on the bench sheet whenever a taxon should be excluded from counts of the number of taxa reported in the sample. This annotation is employed when three conditions co-exist:

The identification is not at the species-level (e.g., Pleustidae or Polydora sp).

And

The reported taxon is represented in the sample by other members of its taxon, which have been identified at lower levels.

And

The taxonomist cannot determine if the specimen is distinct from the other members of its taxon represented in the sample.

4.10.4 It is necessary that the taxonomists make this evaluation during sample analysis (i.e., by annotation of the bench sheet). It cannot be effectively applied after the fact, as there is no way of determining later whether the third criterion

LABORATORY ANALYSIS PROCEDURES FOR SEDIMENT SAMPLES COLLECTED FROM MARINE AND ESTUARINE WATERS FOR BENTHIC INFAUNAL COMMUNITY ASSESSMENT
(Page 9 of 17)

for use was met.

4.10.5 The EXCLUDE notation will be included as part of the electronic data record submitted by each laboratory.

4.10.6 Examples of Use:

Both Dipolydora sp and Dipolydora socialis are reported in a sample and the taxonomist cannot determine if the specimen reported as D. sp is distinct from D. socialis. Exclude (annotate record with **EX**)

An unidentifiable onuphid polychaete is reported as Onuphidae. It is the only member of its family present in the sample. **Do Not Exclude**

Both Modiolus sp and Modiolus capax are reported in a sample. However, the taxonomist is confident that the specimen identified at the genus-level is not M. capax. **Do Not** Exclude

4.11 Temporary "In-House" provisional names are erected for those specimens that a taxonomist considers to be distinctive but cannot match with an existing description. These provisional names act as markers for these taxa, allowing them to be consistently discriminated in the samples for which the taxonomist is responsible. In-house provisional names are supported by a written differential diagnosis (and figures if necessary) sufficient to allow taxonomists in the other participating laboratories to recognize the species. These diagnoses are sent to other taxonomists participating in the survey. The provisional name is formed from the lowest taxon name in which the specimen may be placed with certainty followed by a composite name containing the laboratory's Bight'98 code and a number; for example, Rhachotropis LA2.

4.12 Timely and frequent communication among the taxonomists analyzing the samples will improve the data produced in the survey. An e-mail list-server will be established that will facilitate this communication. All (and only) taxonomists involved in the Bight 98 survey will be members of the list. Messages posted to the list will automatically post to all members, assuring wide and uniform distribution of the contents.

4.13 Appropriate uses of the list server are informing the other members of unusual or newly encountered species, the erection of in-house provisionals, and requests for information or assistance.

4.14 Messages posted to the list-server should always include in the subject line the taxon (if any) to which the posting refers. The body should always begin with the originators name, followed (if appropriate) by the Phylum, Class, Family of topic, then the remainder

LABORATORY ANALYSIS PROCEDURES FOR SEDIMENT SAMPLES COLLECTED FROM MARINE AND ESTUARINE WATERS FOR BENTHIC INFAUNAL COMMUNITY ASSESSMENT

of the text.

4.15 Following identification and enumeration, all the specimens are retained in taxa lots within the sample. Minimally, the material must be segregated into the following 17 taxa lots:

Annelid lots:	**Arthropod lots:**	**Molluscan lots:**
Oligocheata	Ostracoda	Bivalvia
Spionidae	Amphipoda	Gastropoda
Cirratulidae	Decapoda	Misc. Mollusca
Other Polychaetes (by order)	Misc. Arthropoda	
Echinoderm lots:	**Misc. Phyla lots:**	
Ophiuroidea	Cnidaria	Nemertea
Misc. Echinodermata	Nematoda	Other Phyla (a collective lot) This level of separation facilitates the quality control process and eases both the burden of re-analysis resulting from failure of a laboratory to meet the measurement quality objective and the recovery of material during the end-of-survey synoptic review. Further segregation of all polychaetes at the family level has been found useful in some POTW monitoring surveys and is recommended.

4.16 All taxa lots within a sample are provided an internal label with the taxa lot name and station name. These taxa lots are contained in vials and all the lots in a sample aggregated into one or more sample containers. If a taxa lot includes bulky specimens, they may be placed loose in the sample container along with the shell vials containing the remainder of that and other taxa lots. An internal label is placed in each sample container bearing the station name, sampling date, split number (if more than one container is used; e.g., 1of 2). Labels are written in pencil or indelible ink on 100% rag-paper, poly-paper, or other paper suitable for permanent wet labels.

5. QUALITY CONTROL

5.1 The laboratory analysis of infaunal samples for Bight'98 involves four processes: sample washing and preservation, sample sorting, biomass estimation, and organism identification and enumeration. Quality assurance in the form of procedures and standardized reporting requirements are provided in this document for all four processes. Quality control exercises will be implemented at stages for which MQOs have been established (sample sorting, identification and enumeration). These exercises include repeating the procedures at each of these stages for a sub-set of samples. The results will

LABORATORY ANALYSIS PROCEDURES FOR SEDIMENT SAMPLES COLLECTED FROM MARINE AND ESTUARINE WATERS FOR BENTHIC INFAUNAL COMMUNITY ASSESSMENT

be used to determine achievement of the MQOs established for each stage.

5.2 The approach employed to estimate infaunal biomass (measurement of the wet-weight of alcohol-preserved collective taxa lots) does not lend itself to meaningful quality control re-weighing. This variability is a result of the inability to achieve a stable and repeatable amount of preservative within a taxa lot between successive weighings. In addition, there is a tendency for material held in alcohol to lose weight over time. Toleration of the deficiencies of the technique is necessary in order to obtain an estimate of biomass while assuring the preservation of the specimens in a condition that will allow their subsequent identification.

5.3 For the most challenging process, organism identification, additional quality control steps are included in order to foster comparability among the taxonomic data sets produced by the participating laboratories and taxonomists

5.4 In addition, the Benthic Specialist (or designee) may conduct audits of each laboratory while sample analysis is underway to assure that the Bight'98 procedures are being followed.

5.5 Sample Sorting

5.5.1 Quality control of sorting is essential to assure the value of all the subsequent steps in the sample analysis process. An accuracy MQO of 5% (equivalent to 95% removal efficiency) has been set for this stage of the sample analysis. Achievement of this MQO will be determined by re-sorting of 10% of the residue remaining from the original sort.

5.5.2 A standard sorting form is used for tracking the sample. It includes the name of the technician responsible, time required for sorting, comments, and re-sorting results. Re-sorting of samples is employed for quality control of sorting.

5.5.3 A minimum of 10% of all material in Bight'98 samples will be re-sorted to monitor sorter performance and to determine achievement of the MQO of 5%.

5.5.4 Two alternative approaches (described below) are used for re-sorting; the Aliquot method, or the Whole Sample method. The method chosen is at the option of the laboratory. However, a single method must be employed for all samples for which a laboratory provides sorting. The re-sort method used must be noted on the sorting form along with results.

5.5.5 *Aliquot Method:* A representative aliquot of at least 10% of the sample volume

LABORATORY ANALYSIS PROCEDURES FOR SEDIMENT SAMPLES COLLECTED FROM MARINE AND ESTUARINE WATERS FOR BENTHIC INFAUNAL COMMUNITY ASSESSMENT

of every sample processed by each sorter is re-sorted.

5.5.6 *Whole Sample Method:* At least 10% of the samples processed by each sorter are completely re-sorted.

5.5.7 Regardless of the method employed, all re-sorting is conducted by an experienced sorter other than the original sorter.

5.5.8 The responsible supervisor of each participating laboratory is responsible for selection of the method to be used for re-sorting and the unbiased selection of samples and method of obtaining a sample aliquot.

5.5.9 The re-sorting process is to follow the procedures given in §2 of this document.

5.5.10 Percent sorting efficiency is calculated as follows:

Whole Sample Method: $\%_{Efficiency} = 100 * [\#Orgs_{Orig\ sorted}$ divided by $(\#Orgs_{Orig\ sorted} + \#Orgs_{from\ Re\text{-}sort})]$
Aliquot Method: $\%_{Efficiency} = 100 * [\#Orgs_{Orig\ sorted}$ divided by $(\#Orgs_{Orig\ sorted} + \#Orgs_{from\ Re\text{-}sort} * \%_{aliquot})]$

5.5.11 If sorting efficiency is greater than 95%, no action is required. Sorting efficiencies below 95% will require continuous monitoring of that technician until efficiency is improved. If the Whole Sample Method is employed, failure to achieve 95 % sorting efficiency will require re-sorting of all samples previously sorted by that technician.

5.5.12 Organisms found in the re-sort should be included in the results from the sample.

5.5.13 The calculated sorting efficiency is recorded on the Sorting Form for each sample for which QC re-sorting is conducted.

5.5.14 Sample debris left after sorting must be retained by the laboratory responsible for the sorting. It is to be properly labeled and preserved with 70% ethanol. Upon completion of all quality control and assessment steps for the survey, the Benthic Specialist will notify each participating laboratory that the sample debris may be discarded.

5.6 Quality Control of Taxonomic Analysis

LABORATORY ANALYSIS PROCEDURES FOR SEDIMENT SAMPLES COLLECTED FROM MARINE AND ESTUARINE WATERS FOR BENTHIC INFAUNAL COMMUNITY ASSESSMENT
(Page 13 of 17)

5.6.1 The goal of taxonomic analysis for the Bight'98 infaunal survey is species level identification of all macrobenthic organisms collected and an accurate count of each species. This task is complicated by the participation of multiple laboratories and taxonomists in the analysis. Two approaches are taken for providing data quality control. The first is an assessment of each laboratory's accuracy by re-analysis of a subset of samples from each laboratory. The procedures for sample re-analysis are based upon those developed and employed in the Southern California Bight Pilot Project (Montagne & Bergen 1997). The second focuses on ensuring consistent and comparable results among the participating taxonomists through cooperative activities with SCAMIT.

5.6.2 Quality control is provided by the re-identification of 10% of the samples processed by each laboratory. Samples for re-identification are selected randomly from each lab's assigned set of samples by the Bight'98 Benthic Specialist and re-distributed to the other laboratories.

5.6.3 The re-identification will be conducted at participating laboratories and by taxonomists other than those who originally analyzed the samples. The taxonomists conducting the re-identification do not have access to the original results.

5.6.4 Each laboratory's supervisor will be informed by the Benthic Specialist as to which samples are to be re-identified. The laboratory supervisor is responsible for assuring that these samples are made available to the laboratory responsible for re-identification.

5.6.5 The specimens in each sample will be re-identified and enumerated using the procedures given in §4 of this document. Results are reported on the re-analytical laboratory's bench sheet. Upon completion of the re-analysis, the results and original analytical results are exchanged between laboratories.

5.6.6 The supervisors of the laboratories involved compare the original results to those of the re-analysis. All differences in results are listed on the Discrepancy Report. Only discrepancies are reported on this form. A copy of this report is sent to the laboratory responsible for the original analysis.

5.6.7 The two laboratories attempt to reconcile discrepancies. To facilitate this process, two to four SCAMIT/Bight'98 workshops will be scheduled in which taxonomists will jointly meet for discrepancy resolution. Significant discrepancies in count (±5% of original count) are resolved by a third count performed by the re-analytical lab.

5.6.8 The cause and resolution of discrepancies is reported on the Discrepancy

LABORATORY ANALYSIS PROCEDURES FOR SEDIMENT SAMPLES COLLECTED FROM MARINE AND ESTUARINE WATERS FOR BENTHIC INFAUNAL COMMUNITY ASSESSMENT
(Page 14 of 17)

Resolution Report. While completion of this report is the responsibility of the re-analytical laboratory, both labs must work together to reach agreement. If agreement cannot be reached, arguments are presented to the Benthic Specialist for a decision. The Benthic Specialist may seek assistance from SCAMIT members or other experienced taxonomists in reaching a decision.

5.6.9 Once resolution and explanation of all discrepancies has been completed, the Discrepancy Resolution report is sent to the Benthic Specialist along with copies of both laboratory's bench sheets and the Discrepancy Report. Copies of all reports and bench sheets are to be retained by both laboratories.

5.6.10 The Benthic Specialist reviews the results submitted, discusses with the laboratories any issues needing clarification or arbitration.

5.6.11 The Benthic Specialist is responsible for completing the rest of the form, applying the Discrepancy classifications and Resolution codes (see foot of Discrepancy Resolution Report form), and determining the effect of the resolution (increase, decrease, or no change) on the number of taxa and the organism count reported in the original results.

5.6.12 These results are then used to calculate the % error of the original laboratory's analysis. Percent error will be calculated for three aspects of sample analysis; number of taxa discriminated (%Err# Tax), total organism count (%Err# Orgs), and identification accuracy (%ErrID).

5.6.13 The error rates are calculated as follows:

$\%Err_{\#\ Tax} = 100 * [(\#\ Taxa_{Resolved} - \#\ Taxa_{Original}) \text{ divided by } \#\ Taxa_{Resolved}]$
$\%Err_{\#\ Orgs} = 100 * [(\#\ Organisms_{Resolved} - \#\ Organisms_{Original}) \text{ divided by } \#\ Organisms_{Resolved}]$
$\%Err_{ID} = 100 * (\#\ Taxa_{MisID} \text{ divided by } \#\ Taxa_{Resolved})$

The first two aspects provide measures of data quality as relates to parameters such as species richness, abundance, and diversity. The third aspect, identification accuracy, is expressed as percent error in identification of individual taxa. It provides a measure of data quality as a representation of community composition. The calculations only consider errors in the original analysis. The results of these calculations are reported on the Infaunal ID & Enumeration Accuracy Report.

5.6.14 Based upon the results of data quality assessment for the SCBPP, an MQO of 10%, representing the maximum allowable deviation from the "true" value, has been established for number of taxa, total number of organisms, and identification

LABORATORY ANALYSIS PROCEDURES FOR SEDIMENT SAMPLES COLLECTED FROM MARINE AND ESTUARINE WATERS FOR BENTHIC INFAUNAL COMMUNITY ASSESSMENT

accuracy. Each contributing laboratory must strive to avoid exceeding this level of error. The results of this assessment process will provide a measure of the quality of Bight'98 infaunal data, and add to the SCBPP baseline for selection of MQOs in future regional surveys based upon the SCBPP/Bight'98 model.

5.6.15 In addition to providing for an assessment of analytical accuracy, this process provides information for the end-of-survey SCAMIT/Bight'98 synoptic review of the data set compiled from the participating laboratories.

5.6.16 Each participating laboratory must create a voucher collection of all species identified in Bight'98 samples analyzed in that laboratory. These collections are separate from the laboratories' existing voucher collections and will be the source of material from which is drawn a common Bight'98 voucher collection upon completion of the survey. These collections provide material for review during SCAMIT/Bight'98 workshops and the synoptic review of the data upon completion of analysis.

5.6.17 The voucher collections are to contain specimen lots of one or more individuals of each reported taxon. The specimens are to be representative of the taxon. At the taxonomist's discretion, more than one specimen lot may be added to the collection. This is particularly appropriate when differences in specimen maturity, or within-taxon variability need representation. Only those taxa discriminated to the species-level (or stipulated higher level e.g., Oligochaeta) are to be included in the collection. Species-level identification is considered to include provisional species and conditional taxa. Tentative identifications, as indicated by "?" are not to be represented. See the SCAMIT Newsletter (SCAMIT 1986) for protocols and recommendations on provisional and open nomenclature.

5.6.18 Only glass containers are used for the storage of the voucher material, unless specimens are inappropriate for wet storage. Each voucher container should contain an internal label bearing the complete taxon name, author and date. Within the voucher container each specimen lot should be contained within a shell vial closed with cotton or other stopper. Specimens too large to be contained in shell vials may be stored in jars. Each lot is to be accompanied by an internal label bearing the taxon name, station name of sample from which the specimen(s) was removed, a count of the number of specimens in the lot, the analytical laboratory's designation (OC, HY, etc.), and the identifying taxonomist's initials. The use of shell vials for all specimens other than large species will facilitate the consolidation of the voucher collections upon completion of the survey.

5.6.19 Labels are written in pencil or indelible ink on 100% rag-paper, poly-paper, or other paper suitable for permanent wet labels.

LABORATORY ANALYSIS PROCEDURES FOR SEDIMENT SAMPLES COLLECTED FROM MARINE AND ESTUARINE WATERS FOR BENTHIC INFAUNAL COMMUNITY ASSESSMENT

5.6.20 Taxonomists from the participating laboratories are required to participate in special SCAMIT/Bight'98 workshops. Workshops prior to the sampling period focus on the taxonomy of groups requiring particular review to promote uniform treatment in the upcoming survey. The workshops provide training, pooling of regional resources, and designation of the local expert(s) to be called upon for assistance during sample analysis.

5.6.21 Based upon these workshops and the results of the SCBPP quality control results, a limited number of taxa may be selected for special treatment. These are groups for which prior experience leads us to believe consistent identification will not be possible unless all the collected material is identified by a single taxonomist or small team of taxonomists. During regular sample analysis, all members of a taxon selected for this specialized treatment will be identified at a standard collective level (e.g., class or other high-level category), counted and segregated into a lot for subsequent processing by the specialist(s). Details of this process will be developed during the SCAMIT/BIGHT98 workshops.

5.6.22 After sample analysis has begun, SCAMIT/Bight'98 workshops continue at least monthly to address taxonomic problems arising during analysis of the Bight'98 samples. At these meetings, diagnoses of any "in-house" provisional taxa erected by any of the laboratories will be distributed to the other participants and assistance sought to resolve their identity. SCAMIT provisional species names will be provided for those found to be or suspected of being new species.

5.6.23 The series of SCAMIT/Bight'98 workshops culminates in a synoptic review of the data set compiled from all participating laboratories, and investigation of possible inconsistencies revealed in that process (including examination of voucher specimens or sample lots as needed for resolution). This review also draws upon the results of the quality control re-analysis of 10% of the samples analyzed by each laboratory.

6. RECORD KEEPING AND PROCEDURAL RESPONSIBILITY

6.1 Each laboratory must be responsible for maintaining thorough and complete records through all stages of the sample analysis and QC procedures. Each laboratory will employ its own bench sheet for taxonomic analysis. For the Bight'98 infaunal survey, certain standard forms of notation are employed with the taxonomist's bench sheet that assure that all labs collect the required information in uniform fashion. Standardized forms are used for sorting and all QC checks. Each participating laboratory will retain its taxonomic bench sheets and voucher sheets. All QC reports are to be submitted to the Benthic Specialist upon completion of sample analysis. Copies of all these documents are to be retained by the individual laboratories. Analytical results are to be transmitted to the

LABORATORY ANALYSIS PROCEDURES FOR SEDIMENT SAMPLES COLLECTED FROM MARINE AND ESTUARINE WATERS FOR BENTHIC INFAUNAL COMMUNITY ASSESSMENT
(Page 17 of 17)

Information Management officer.

6.2 The laboratory supervisor is responsible for assuring that all steps in the process of analyzing infaunal samples follow Bight'98 procedures and that all QC steps are completed and documented. The supervisor must implement any specified corrective actions resulting from QC protocols. He or she is also responsible for preparing their data and documents for transmission to the Information Management Officer in the proper form. All data entry must be subject to the established transcription error checking procedures within the originating laboratory.

7. REFERENCES

Montagne, D. E. & M. Bergen. 1997. Quality Control and Assessment of Infaunal Identification and Enumeration: The SCBPP Experience. Southern California Research Project Annual Report 1996. Westminster, CA. pp 147-154.

SCAMIT. 1986. Protocols and Recommendations for the Use of Open Nomenclature.

SCAMIT Newsletter, May 1986, vol. 5 No. 2.

SCAMIT. 1998. A Taxonomic Listing of Soft Bottom Macro- and Megainvertebrates from Infaunal and Epibenthic Monitoring Programs in the Southern California Bight. Edition 3. SCAMIT, San Pedro, CA. 167 pp.

8. DATA FORMS

This section includes examples of the data forms used for the laboratory analysis and QC of Bight'98 infaunal samples. They are (HTML active items below):

- Infaunal Sorting Sheet and Sorting Quality Control Report
- Infaunal Biomass Sheet
- Infaunal Analysis QC Discrepancy Report (a multi-page form)
- Infaunal Analysis QC Discrepancy Resolution Report (a multi-page form)
- Infaunal Id & Enumeration Accuracy Report