Southern California Bight
2003 Regional Marine Monitoring Survey
(Bight'03)

Macrobenthic (Infaunal) Sample Analysis Laboratory Manual

Prepared by:
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Prepared for:
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SOUTHERN CALIFORNI
A BIGHT 2003 REGIONAL
MONITORING PROGRAM

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INTRODUCTION

This document describes laboratory procedures to be followed in the analysis of macrobenthic (infaunal) samples collected for the Southern California Bight 2003 Regional Marine Monitoring Program (Bight'03). 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) and the Southern California Bight 1998 Regional Monitoring Program (Bight'98). Some modifications have been made to ensure data comparability, to facilitate the coordination of the quality control steps required for the Bight'03 infaunal survey, and to meet the requirements of the B'03 Information Management Plan. It is the responsibility of each participating laboratory's supervisor to assure:

- That the detailed procedures described in this manual are followed during sample processing and analysis,
- All Quality Control (QC) steps are implemented,
- Data submissions conform to the stipulated data standards,
- Schedules are met for sample analysis, QC , data submission, and
- 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.

All stages of infaunal sample processing and analysis, following receipt of samples in the laboratory including QC and data submission standards are described in this manual. In overview, the process (and the relevant sections) consists of the following tasks and activities:

1) Sample Treatment and Storage: the sample is washed free of fixative and transferred to an alcohol solution for processing and/or storage (**Section 1**),
2) Sorting: all organisms are removed from the debris contained in the sample and sorted into taxa groupings to facilitate subsequent taxonomic analysis (**Section 2**),
3) Taxonomic Analysis: all specimens in the samples are identified and counted (**Section 3**),
4) Data Submission: resulting data are loaded to an electronic data file compliant with this manual and the B'03 Information Management Plan and submitted to the project Information Management officer (**Section 4**).
5) Quality Control: QC is required for steps 2 and 3 (Section 5) to ensure data consistency. QC consists of reanalysis of selected samples and taxonomist participation in workshops. Results of this process are used to determine whether the measurement quality objectives (MQOs) established for each of these steps are met.
6) Record keeping and Procedural responsibilities are described in **Section 6** and examples of Forms to be used during processing and QC are in **Section 8.**
7) Taxonomist qualification criteria are described in **Appendix A**.

In addition, taxonomists must participate in a series of workshops jointly sponsored by Bight'03 and the Southern California Association of Marine Invertebrate Taxonomists (SCAMIT) which will focus on taxonomic problems arising during analysis of the Bight'03 samples. These workshops culminate in a synoptic review of the data set compiled from all participating laboratories.

Copies of this manual are available in Portable Document Format (pdf) on the web site of the Southern California Coastal Water Research Project (SCCWRP) (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 and fecal pellets that may have broken down during fixation and, 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 all stages of Bight'03 infaunal samples is a 70% solution of ethanol. **Denatured alcohol is not permitted for this purpose**. No rose bengal for staining organisms is to be used.

1.4 It is recommended that the preservative be buffered with marble chips, especially if the ethanol produced by industrial distillation rather than fermentation.

1.5 Procedure

1.5.1 Select an appropriate sieve, which will be a 0.5mm or smaller sieve, and examine the mesh for holes and adhering organisms. Working under a fume hood and with eye protection, decant the fixative through the clean and intact sieve.

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

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

1.5.4 Using a spatula and wash bottle containing preservative, transfer the sample back to the sample container, top the sample with preservative (70% ethanol), and tightly affix the lid.

1.5.5 Place an internal label in each sample container bearing the station name, sampling date, split number (if more than one container is used; *e.g.,* 1 of 2). Labels are to be written in pencil or indelible ink on 100% rag-paper, poly-paper, or other paper suitable for permanent wet labels.

1.5.6 After each sample is washed, closely examine the sieve to assure that all organisms have been removed then thoroughly rinsed to avoid cross

contamination of subsequent samples.

1.5.7 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

1.5.8 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 (debris) 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'03 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'03.

2.2.2 Begin the sorting process by filling out a Bight'03 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 sample. Make sure you have all jars composing the sample.

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

2.2.3 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 supervisor is to contact the Bight'03 Benthic Committee Chairperson. The decision whether to allow sorting by aliquot will be made by the Benthic Committee.

2.2.4 ELUTRIATION**.** If a sample is primarily coarse sand, 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.

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

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

2.2.4.3 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.

2.2.4.4 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.

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 taxonomic lots for subsequent taxonomic analysis. Each laboratory will determine the taxonomic level of sorting adequate to their needs for subsequent sample analysis by their taxonomists.

2.2.7 Remove all individual organisms and fragments from the sample with the exception of nematodes, 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 and identity 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), sampling date and depth. Place an internal label in each sample container bearing the station name, sampling date, depth, 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. TAXONOMIC ANALYSIS

3.1 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.

3.2 The goal of the Bight'03 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:

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

3.3 The number of organisms reported must account for all organisms in a sample alive at the time of collection. A corollary goal is to not count any individual more than once. Inevitably, samples contain fragments of organisms. 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. Also, care must be taken to avoid reporting empty mollusk shells or crustacean molts in the data.

3.4 The goal of the survey is to describe the macroinvertebrate infauna and epifauna living in soft-bottom habitats. Hard-bottom epifaunal organisms may occur incidentally in samples, particularly in settings where samples are collected immediately adjacent to hard structure (*e.g.,* in harbors near piers). As any records of these incidental contaminants would not be included in the analytical use of the data, these specimens are not to be counted nor included in the submitted survey data. Their presence may be noted on the bench sheets.

3.5 Attached parasites and other epibionts may be noted on the bench sheet as present but are not to be reported in the submitted survey data. Ectoparasites of fish such as cymothid isopods, which may be temporary members of the benthic community, are counted and reported in the submitted survey data.

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

3.7 Nomenclature and orthography follows that used in the Edition 4 of the Southern California Association of Marine Invertebrate Taxonomists' taxonomic listing (SCAMIT, 2001). This list represents a consensus for standard usage of taxa names in POTW monitoring programs in the Southern California Bight. An electronic version of a species

list derived from that publication will be made available to the participating organizations submitting data.

3.8 The name used to represent a taxon should be that listed in the SCAMIT Taxonomic listing unless the name listed has been supplanted by a new synonym in which case the currently accepted synonym is to be used.

3.9 Taxonomists are to employ two standard notations (*Voucher* and *Exclude*) for the annotation of their bench sheets. While other non-standard notation may also be used, the use of these standard notations is required where applicable. In addition, both the Voucher and Exclude codes will be included as part of the electronic data record. See the Bight'03 Information Management Plan for the proper form for these fields for data submission.

3.10 Voucher Notation

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

3.10.2 Purpose: To note the removal of specimens from a sample for use as Bight'03 vouchers. Use of this notation on the bench sheet 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. Its inclusion in the electronic data summation allows a complete list of Bight'03 vouchers to be extracted from the data.

3.10.3 Rule of Use: Removal of any specimens from a sample to the B'03 voucher collection is clearly noted on the bench sheet by means of the Voucher notation.

3.10.4 In addition to the voucher specimens required for the B'03 Voucher Collection (see 5.6.16-20 below), individual labs or taxonomists may remove no more than two specimens of each taxon for their own voucher collections. The removal of this material must also be clearly noted (<u>by means other than the voucher notation</u>) on the bench sheet in order to account for their effect on quality control re-analysis. The following would satisfy the requirement for clear notation:

"V-2, HY-1 voucher"

indicating 2 specimens removed to the B'03 voucher collection and 1 specimen to Hyperion's collection.

3.10.5 The Voucher notation will be included as part of the electronic data record submitted by each laboratory. See the Bight'03 Information Management Plan for the proper format for its inclusion in the data file. <u>Do not</u> include a electronic record of any other specimens removed to the lab or taxonomist's voucher collections.

3.11 Exclude Notation

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

3.11.2 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.

3.11.3 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 the same 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.

3.11.4 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 for use was met.

3.11.5 The Exclude notation will be included as part of the electronic data record submitted by each laboratory. See the Bight'03 Information Management Plan for the proper format for its inclusion in the data file.

3.11.6 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 on bench sheet 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

3.12 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 two-character code (see below) and a number; for example, *Rhachotropis* sp LA2 or Ampharetidae SD1. Note there is no space between the agency code and the identifying number.

Lab	*Code*	*Lab*	*Code*
ABC Labs	AB	MEC	ME
Hyperion, EMD	HY	CSDMWWD	SD
LACSD	LA		

3.13 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 taxonomists involved in the Bight 03 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.

3.14 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.

3.15 Messages posted to the list-server should always include in the subject line the critical topic taxon (if any) to which the posting refers followed by a referent higher taxonomic category in parentheses. For example:

Balanoglossus (Hemichordata)

or

Guernea ME1 (Gammaridea: Dexaminidae)

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

Annelid lots:
- Oligochaeta
- Spionidae
- Cirratulidae
- Misc. Polychaetes

Arthropod lots:
- Ostracoda
- Amphipoda
- Decapoda
- Misc. Arthropoda

Molluscan lots:
- Bivalvia
- Gastropoda
- Misc. Mollusca

Echinoderm lots:
- Ophiuroidea
- Misc. Echinodermata

Misc. Phyla lots:
- Cnidaria
- Nemertea
- 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. In addition, any taxon subject to specialty taxonomic treatment (see 5.6.22 below) is to be segregated into a lot for delivery to the designated specialist.

Further segregation of all polychaetes at the family level has been found useful in some POTW monitoring surveys and is recommended.

3.17 All taxa lots within a sample are provided an internal label with the program designation (*i.e.,* B'03), taxa lot name, station name and depth. These taxa lots are contained in shell vials and all the lots in a sample aggregated into one or more sample containers. Shell vials are to be no smaller than ½ dram capacity and are stoppered with cotton. 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 program designation (*i.e.,* B'03), station name, sampling date, depth, and 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.

§ 4. DATA SUBMISSION: The Form of Taxonomic Names

4.1 All data submissions must meet the formatting requirements of the Bight'03 Information Management Plan.

4.2 In particular, it is essential that all taxon names be standardized in spelling and form. Because the "species" field is one of the key fields for defining a unique record, exactitude is required.

4.3 To minimize the problem of variants, a standard for the spelling and formation of names has been specified prior to the survey. This standard is based on the 4th edition of the Southern California Association of Marine Invertebrate Taxonomists' taxonomic listing (SCAMIT, 2001). An electronic version of a species list derived from that publication will be made available to the participating organizations submitting data.

4.4 The name used to represent a taxon should be that listed in the SCAMIT Taxonomic listing unless the name listed has been supplanted by a new synonym in which case the currently accepted synonym is to be used.

4.5 The following examples of data submission problems from Bight'98 are included to emphasize the importance of adhering to the Information Management Plan requirements for submission of taxonomy-based data.

4.5.1 The species field is to contain taxon names only. Do not include citation of authorship, comments or other information

As Submitted	***Should Have Been***
Anthozoa, unid.	Anthozoa
Bugula neritina (colonial)	Bugula neritina
Enopla sp A SCAMIT 1995	Enopla sp A
Heteroserolis n. sp.?	Heteroserolis sp
Tubulanus polymorphus/pellucidus	Tubulanus polymorphus
frags only	*not submitted*

4.5.2 The species field is to contain formal scientific taxa names only. Do not use common names or anglicized forms

As Submitted	***Should Have Been***
Cirriped	Cirripedia
megalopa	Decapoda (*not the larval stage*)
fish	*a particular fish taxon (at any level)*

4.5.3 The form (spelling, punctuation) of the names are to follow the SCAMIT Taxonomic listing. Note that the SCAMIT list avoids all forms of punctuation (other than parentheses around subgenus names) within a taxon name.

As Submitted	***Should Have Been***
Scoloplos "armiger"	Scoloplos armiger Cmplx
Semele sp.	Semele sp
Aphelochaeta spp	Aphelochaeta sp
Prionospio jubata	Prionospio (Prionospio) jubata

4.5.4 In forming or using provisional names based upon the two character agency code, do not include a space between the agency code and the number

As Submitted	***Should Have Been***
Anobothrus sp LA 1	Anobothrus sp LA1
Malmgreniella sp SD 3	Malmgreniella sp SD3

4.6 ENCOUNTERED SPECIES LIST: All submissions are to be accompanied by an encountered species list providing the taxon name and, for species level names, authorship citation. These lists will facilitate the recognition of variant forms within the compiled data set and, more importantly, the cases of potential or real homonymy or synonymy. A comments column is provided to provide optional information that may be of value in evaluating the list entries.

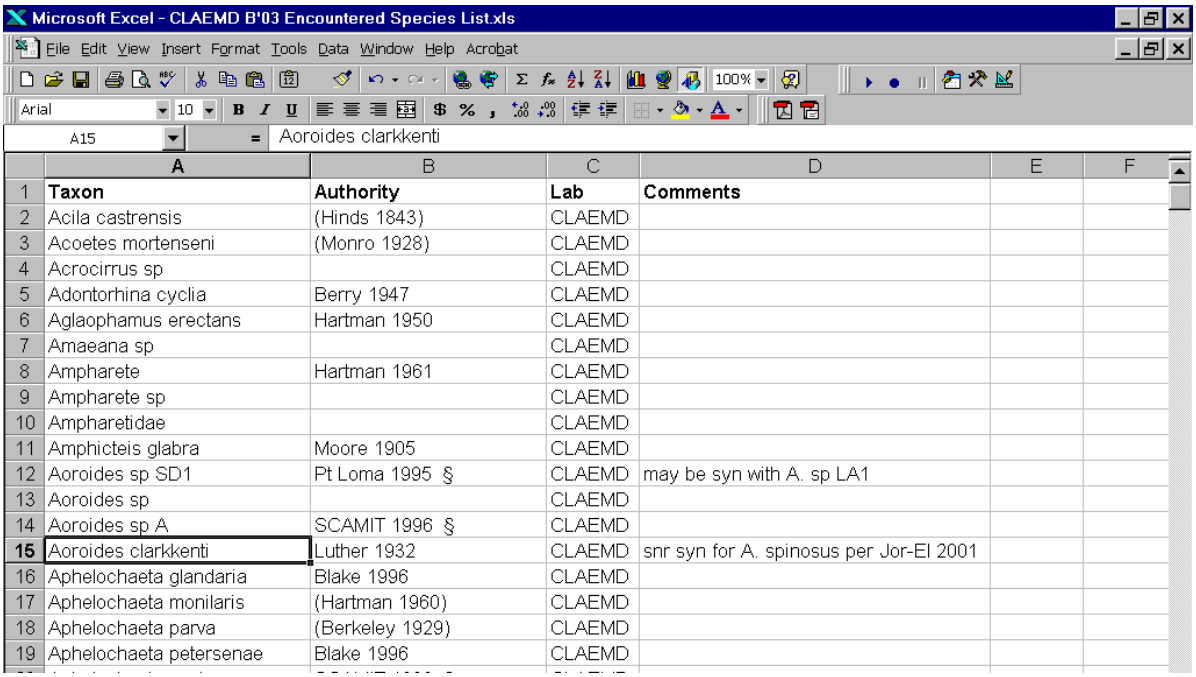
4.6.1 The encountered species list should contain every unique taxon name occurring within the data being submitted.

4.6.2 The encountered species list should be in the form of a three column Excel worksheet with the following format:

Column A = Taxon
Column B = Authority (*for species-level taxa*)
Column C = Lab (*the B'03 Information Plan agency code*)
Column D = Comments

4.6.3 The list should be sorted alphabetically by taxon name

4.6.4 Figure: An example of the required Encountered Species List.



Microsoft Excel - CLAEMD B'03 Encountered Species List.xls

File Edit View Insert Format Tools Data Window Help Acrobat

Arial 10 100%

A15 = Aoroides clarkkenti

	A	B	C	D	E	F
1	**Taxon**	**Authority**	**Lab**	**Comments**		
2	Acila castrensis	(Hinds 1843)	CLAEMD			
3	Acoetes mortenseni	(Monro 1928)	CLAEMD			
4	Acrocirrus sp		CLAEMD			
5	Adontorhina cyclia	Berry 1947	CLAEMD			
6	Aglaophamus erectans	Hartman 1950	CLAEMD			
7	Amaeana sp		CLAEMD			
8	Ampharete	Hartman 1961	CLAEMD			
9	Ampharete sp		CLAEMD			
10	Ampharetidae		CLAEMD			
11	Amphicteis glabra	Moore 1905	CLAEMD			
12	Aoroides sp SD1	Pt Loma 1995 §	CLAEMD	may be syn with A. sp LA1		
13	Aoroides sp		CLAEMD			
14	Aoroides sp A	SCAMIT 1996 §	CLAEMD			
15	Aoroides clarkkenti	Luther 1932	CLAEMD	snr syn for A. spinosus per Jor-El 2001		
16	Aphelochaeta glandaria	Blake 1996	CLAEMD			
17	Aphelochaeta monilaris	(Hartman 1960)	CLAEMD			
18	Aphelochaeta parva	(Berkeley 1929)	CLAEMD			
19	Aphelochaeta petersenae	Blake 1996	CLAEMD			

§ 5. QUALITY CONTROL

5.1 The laboratory analysis of infaunal samples for Bight'03 involves three processes: sample washing and preservation, sample sorting, and organism identification and enumeration. Quality assurance in the form of procedures and standardized reporting requirements are provided in this document for all three 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 be used to determine achievement of the MQOs established for each stage.

5.2 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

5.3 In addition, the Benthic Committee Chairperson (or designee) may conduct audits of each laboratory while sample analysis is underway to assure that the Bight'03 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.

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 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, an experienced sorter other than the original

sorter conducts all re-sorting.

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:

$$\%_{\text{Efficiency}} = 100 * [\#\text{Orgs}_{\text{Orig sorted}} \div (\#\text{Orgs}_{\text{Orig sorted}} + \#\text{Orgs}_{\text{from Re-sort}})]$$

Aliquot Method:

$$\%_{\text{Efficiency}} = 100 * [\#\text{Orgs}_{\text{Orig sorted}} \div (\#\text{Orgs}_{\text{Orig sorted}} + \#\text{Orgs}_{\text{from Re-sort}} * \%_{\text{aliquot}})]$$

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 The laboratory responsible for the sorting must retain sample debris left after 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 Committee Chairperson (or designee) will notify each participating laboratory that the sample debris may be discarded.

5.6 Quality Control and Quality Assessment of Taxonomic Analysis

5.6.1 The goal of taxonomic analysis for the Bight'03 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 SCBPP and in the Bight'98 survey. The second focuses on ensuring consistent and comparable results among the participating taxonomists through cooperative activities under the aegis of 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'03 Benthic Committee Chairperson (or designee) 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 Committee Chairperson (or designee) 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 in a timely manner.

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'03 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 are reported on the Discrepancy 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 Bight'03 Benthic Committee Chairperson (or designee) for a decision. The Chairperson 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 Committee Chairperson (or designee) 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 Committee Chairperson (or designee) reviews the results submitted,

discusses with the laboratories any issues needing clarification or arbitration.

5.6.11 The Benthic Committee Chairperson (or designee) 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 ($\%Err_{ID}$).

5.6.13 The error rates are calculated as follows:

$$\%Err_{\#\ Tax} = 100 * [(\#\ Taxa_{Resolved} - \#\ Taxa_{Original}) \div \#\ Taxa_{Resolved}]$$

$$\%Err_{\#\ Orgs} = 100 * [(\#\ Organisms_{Resolved} - \#\ Organisms_{Original}) \div \#\ Organisms_{Resolved}]$$

$$\%Err_{ID} = 100 * (\#\ Taxa_{MisID} \div \#\ Taxa_{Resolved})$$

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 and Bight'98, 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 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'03 infaunal data, and add to the baseline for selection of MQOs in future regional surveys based upon the this 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'03 Synoptic Data 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'03 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'03 voucher collection upon completion of the survey. These collections provide material for review during

SCAMIT/Bight'03 workshops and the Synoptic Data Review 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 stopper. Shell vials shall have a minimum capacity of ½ dram. 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.

5.6.20 After the vouchering needs of the Bight'03 survey are met, individual labs or taxonomists may remove a limited number of specimens (no more than 2 per species) for their own voucher collections. This activity is separate from and subordinate to the B'03 vouchering requirement. Unique specimens must be reserved for the B'03 voucher collection.

5.6.21 Taxonomists from the participating laboratories are required to participate in special SCAMIT/Bight'03 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.

5.6.22 Based upon these workshops and the results of the Bight'98 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). These data will be included in the sample submission using the specified standard collective taxon name as a placeholder pending results of the specialized analysis. Each placeholder record shall be marked by the insertion of the value "S" in the qualifier field of the data file (see B'03 Information Management Plan). The individual labs are <u>not</u> responsible for incorporating the results of the specialized analysis into the data. This task will be the responsibility of the Benthic Committee Chairperson (or designee) and will take place following compilation of a data set from all data submitted by the participating laboratories.

5.6.23 After sample analysis has begun, SCAMIT/Bight'03 workshops continue at least monthly to address taxonomic problems arising during analysis of the Bight'03 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.

5.6.24 The series of SCAMIT/Bight'03 workshops culminates in a Synoptic Data Review of the data set compiled from the submissions of 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 & 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'03 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 Committee Chairperson (or designee) upon completion of sample analysis. To insure against loss of documents, copies of all these documents are to be retained by the individual laboratories.

6.2 The laboratory supervisor is responsible for assuring that all steps in the process of analyzing infaunal samples follow Bight'03 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. Analytical results are to be transmitted to the Information Management officer in electronic data files that conform to Bight'03 data submission formats and standards as described in the Information Management Plan. It is the submitting laboratory's responsibility to see that these standards are met.

§ 7. REFERENCES

SCAMIT. 1986. *Protocols and Recommendations for the Use of Open Nomenclature*. SCAMIT Newsletter, May 1986, vol. 5 No. 2.

SCAMIT. 2001. *A Taxonomic Listing of Soft Bottom Macro- and Megainvertebrates from Infaunal and Epibenthic Monitoring Programs in the Southern California Bight.* Edition 4. Southern California Assoc. of Marine Invertebrate Taxonomists, San Pedro, CA. 192 pp.

§ 8. DATA FORMS

This section includes examples of the data forms used for the laboratory analysis and QC of Bight'03 infaunal samples. They are:

Infaunal Sorting Sheet and Sorting Quality Control Report

Infaunal Analysis QC: Discrepancy Report (*a multi-page form*)

Infaunal Analysis QC: Discrepancy Resolution Report (*a multi-page form*)

Infaunal Id & Enumeration: Accuracy Report (*for use by Benthic Committee Chair or Designee*)

Forms are available on the web site of the Southern California Coastal Water Research Project (http://sccwrp.org in Portable Document Format (pdf).

APPENDIX A

TAXONOMIST QUALIFICATION

FOR BIGHT'03

MACROBENTHIC (INFAUNAL) SAMPLE ANALYSIS

Prepared by:
Bight'03 Benthic Committee

Prepared for:
Commission of Southern California Coastal Water Research Project
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June 2003

INTRODUCTION

The Bight'03 macrobenthic survey is a multi-agency, regional survey of shelf, slope and nearshore soft-bottom infaunal communities within the Southern California Bight. The survey design, field and laboratory procedures, and QA/QC plan are based upon the experience gained during the 1994 Southern California Bight Pilot Project (SCBPP) and the 1998 Southern California Bight Regional Monitoring Program (Bight'98) infaunal surveys. As in these surveys, the Bight'03 infaunal survey involves the integration of data produced by a large number of taxonomists into a single data set. These taxonomists are employed or contracted by several different agencies participating in the Bight'03 project. As was discovered during the SCBPP and Bight'98, the difficulty of assuring accurate and consistent results in a large scale infaunal survey is compounded by the differences in the expertise, experience and opinion of the participating taxonomists. To minimize the effect of these problems on the survey results, detailed quality assurance plans, including quality control exercises and quality assessments relative to specific quality objectives for taxonomic analysis were established. Similar steps will be employed in the Bight'03 infaunal survey.

In order to assure that the data produced by the Bight'03 infaunal survey meets the standards set during the previous two regional surveys, it is essential that all participating taxonomists have the expertise and experience necessary to produce data of comparable quality. Qualification criteria have been established to assure that the taxonomists participating in the Bight'03 are capable of meeting that standard. Agencies or their contractors employing taxonomists who did not perform analysis of infaunal samples for the SCBPP or Bight'98 are required to assure that their taxonomists meet the qualifying criteria prior to participation in the Bight'98 infaunal survey. The two criteria are:

- Candidate taxonomists who will be working under the direct oversight and guidance of an experienced taxonomist who analyzed samples in either the SCBPP or Bight'98 are considered to meet the standard for Bight'03.

- Candidate taxonomists who will be not be working under the direct supervision and guidance of an experienced taxonomist who analyzed samples in either the SCBPP or Bight'98 must complete and pass a qualification exercise prior to acceptance as a taxonomist for Bight'03.

In summary, the exercise is based upon that used as quality control and assessment in the SCBPP and Bight'98 Surveys(Montagne & Bergen 1997, Ranasinghe et al. 2003). The candidate taxonomist will analyze two lots of specimens from samples previously analyzed by SCBPP/Bight'98 taxonomists. The results of the analysis are compared to those of the original taxonomist and the discrepancies classified. Each discrepancy found to be the result of error on the part of the candidate taxonomist will be tallied. The effect upon the number of taxa, organism count, and the accuracy of identification will be determined and a percent error of analysis calculated. The candidiate taxonomist must be able to meet the measurement quality objective (MQO) of 10% for each of the parameters.

The qualification criteria and procedure for the pre-survey qualification exercise are detailed below.

TAXONOMIST QUALIFICATION CRITERIA

A1. Each Agency or its contractor will provide the chairperson of the Bight'03 Benthic Committee a list of the taxonomists who will be employed for sample analysis, along with the taxonomic group(s) for which each will be responsible.

A2. Those taxonomists who have provide infaunal sample analysis in the SCBPP and/or Bight'98 surveys are qualified to participate in Bight'03 sample analysis

A3. Any taxonomist proposed who did not participate in the SCBPP or Bight'98 infaunal sample analysis will be considered a candidate taxonomist and must meet either of two criteria to be allowed to provide sample analysis for Bight'03.

A4. Criteria

A4.1 Candidate taxonomists who will be working under the direct oversight and guidance of an experienced taxonomist who analyzed samples in either the SCBPP or Bight'98 are considered to meet the standard for Bight'03.

A4.1.1 In this context, direct oversight and guidance means they are physically co-located and actively engaged with the taxonomist providing oversight and guidance.

A4.1.2 Oversight and guidance shall include interactive training and review of identifications and sample processing procedures.

A4.2 Candidate taxonomists who will be not be working under the direct oversight and guidance of an experienced taxonomist as defined above must complete and pass a qualification exercise prior to acceptance as a taxonomist for Bight'03.

A5 Qualification Exercise Procedure

A5.1 The purpose of the exercise is to demonstrate the candidate taxonomist's familiarity with the shelf/slope-depth infauna of the Southern California Bight and ability to produce results compatible with those of the other taxonomists who will be performing sample analysis for the Bight'03 infaunal survey.

A5.2 Each candidate is required to analyze (identify and enumerate) two taxa lots for each taxonomic group for which they will be responsible.

A5.3 The taxa lots will come from infaunal samples collected from the SCB Shelf by methods to be used in the Bight'03 survey. For instance, a candidate to perform polychaete identifications will be provided two polychaete lots, each containing all polychaetes from a single 0.1 sq. meter Van Veen Grab, screened on a 1.0 mm

mesh sieve.

A5.4 These samples will have been previously analyzed by taxonomists who participated in the Bight'98 survey.

A5.5 The samples will be provided to the candidates through their employer by the Bight'03 Benthic Committee. The analysis must be completed and the results returned in a timely manner.

A5.6 In conducting the analysis the candidate taxonomist is to follow the conventions below:

A5.6.1 Identify all specimens to the lowest practicable level and provide an accurate count of each identified taxon. Species-level identifications are expected in most cases.

A5.6.2 Fragments of bilaterally symmetrical organisms are to 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 are to be identified and counted.

A5.6.3 Report results on the standard taxonomy data sheets used in the laboratory for recording of identifications and counts.

A5.6.4 For each name reported in the results create a taxa lot containing all specimens represented by that name. (*e.g.,* all *Photis brevipes* in a sample are to be aggregated into a single lot). These taxa lots are to contain an internal label providing the sample name and the taxon contained in the lot. Non-countable fragments may be aggregated into a fragments lot.

A5.6.5 Aggregate all taxa lots from a single sample site (sample name) into a single container provided with an internal label identifying the sample.

A5.6.6 All specimens are to be maintained in a preservative solution of 70% non-denatured ethanol.

A5.6.7 Labels are to be written in pencil or indelible ink on 100% rag-paper, poly-paper, or other paper suitable for permanent wet labels.

A5.6.8 Upon completion of analysis, return the results and all sample material (sorted into taxa lots) to the Benthic Committee Chairperson who will review the results, comparing them to the results of the original analysis.

A5.7 Following procedures based upon those used during the previous regional surveys, the Benthic Committee Chairperson (or designee) will classify all

discrepancies and calculate the % error of analysis. Percent error will be calculated for three aspects of sample analysis; number of taxa discriminated, organism count, and identification accuracy.

A5.8 The results of the exercise will be assessed by an *ad hoc* committee made up of the Chairperson of the Bight'03 Benthic Committee and selected members of SCAMIT with previous experience in the conduct of multi-laboratory taxonomic analysis. This committee will determine whether a candidate taxonomist is capable of meeting the data quality objectives of the Bight'03 infaunal survey. Members selected for the *ad hoc* committee will not be in a position to benefit from the conclusions of the committee.

A5.9 Based upon this assessment, the committee will provide a report to the Bight'03 Coastal Ecology Planning Committee recommending the acceptance or rejection of the candidate taxonomist. A negative recommendation will be accompanied by the reasons for that judgment and what steps, if any, should be taken to remedy the deficiency.

Montagne, D.E. and M. Bergen. 1997. Quality Control and Assessment of Infaunal Identification and Enumeration: The SCBPP Experience. pp 147-154. *In*: Weisberg, S.B., C. Francisco, D. Hallock, (eds.). *Southern California Coastal Water Research Project Annual Report 1996.* Westminster, CA.

Ranasinghe, J.A., D.E. Montagne, R.W. Smith, T.K. Mikel, S.B. Weisberg, D. Cadien, R. Velarde and A. Dalkey. 2003. *Southern California Bight 1998 Regional Monitoring program: VII. Benthic macrofauna.* Southern California Coastal Water Research Poject. Westminster, CA. 91 p + 9 Appendices.