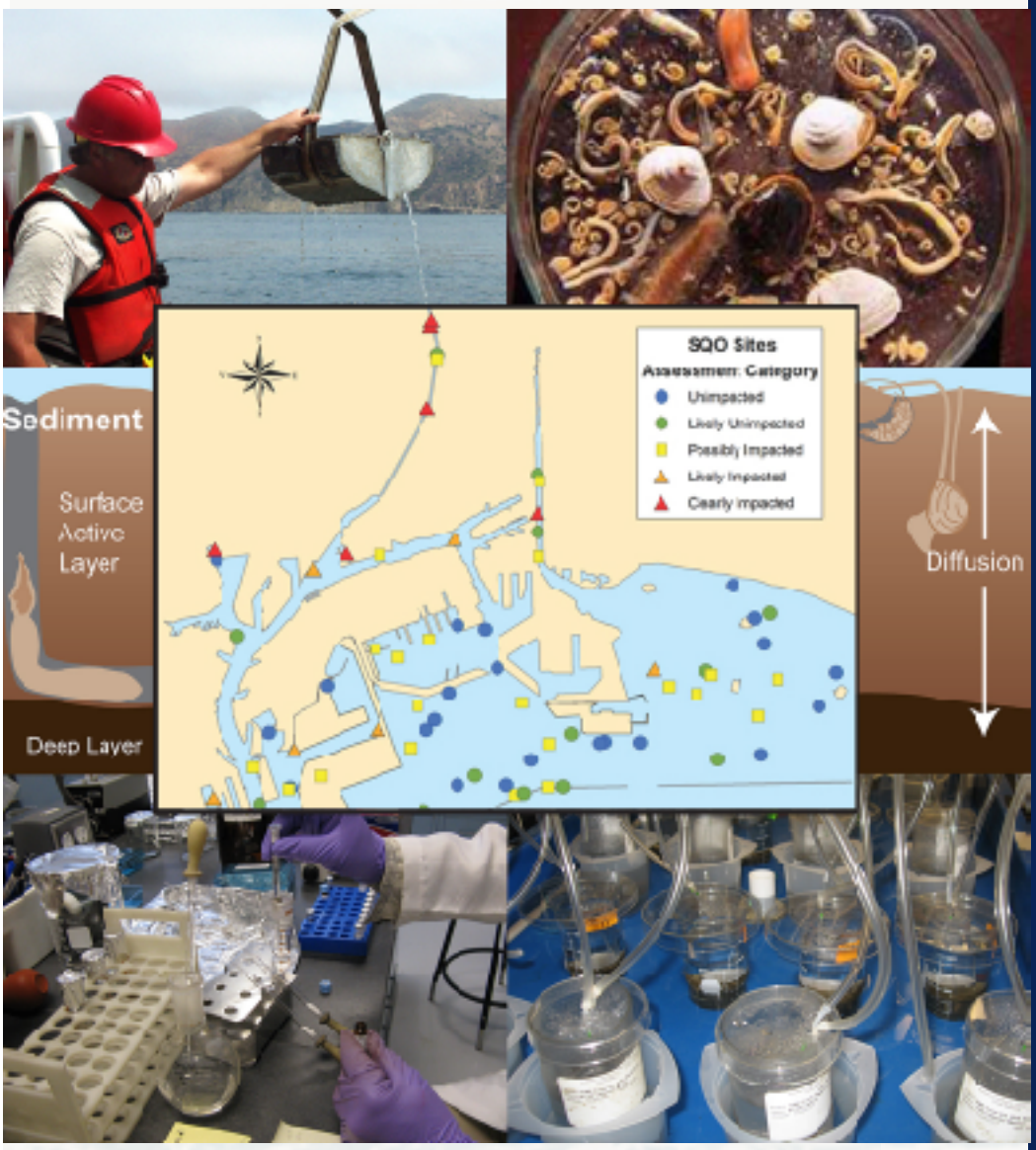
# SEDIMENT QUALITY ASSESSMENT DRAFT TECHNICAL SUPPORT MANUAL

This document was prepared by the State Water Board's technical team to provide end users with guidance for the application of the direct effects tools identified in the State Water Board's Water Quality Control Plan for Enclosed Bays and Estuaries - Part 1 Sediment Quality. This draft document consists of recommended approaches and does not represent regulation or direction from the State Water Board.




*Steven M. Bay*
*Darrin J. Greenstein*
*J. Ananda Ranasinghe*
*Dario W. Diehl*
*A. Elizabeth Fetscher*

*Southern California Coastal Water Research Project*

Technical Report 582 - May 2009

# Sediment Quality Assessment
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Steven M. Bay
Darrin J. Greenstein
J. Ananda Ranasinghe
Dario W. Diehl
A. Elizabeth Fetscher

Southern California Coastal Water Research Project
3535 Harbor Blvd., Suite 110
Costa Mesa, CA 92626
***www.sccwrp.org***

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The authors wish to acknowledge the combined efforts of the many organizations and their staff that have worked cooperatively over the years to standardize and improve sediment quality assessment methods within California. Substantial portions of this document have drawn from the quality assurance plans and laboratory methods documents produced by these groups as part of various regional monitoring and assessment programs, such as the Bay Protection and Toxic Cleanup Program (BPTCP), San Francisco Bay Regional Monitoring Program (RMP), southern California Bight regional monitoring surveys, Surface Water Ambient Monitoring Program (SWAMP), Environmental Monitoring and Assessment Program (EMAP), and numerous compliance monitoring programs. The experience and results obtained from these programs have mad a major contribution to the development of this manual.

We also wish to thank the many scientists and environmental managers that have contributed to the development and review of the methods described here as part of the Sediment Quality Objectives (SQO) program; including members of the technical team, Advisory Committee, Agency Coordination Committee, and Scientific Steering Committee. Special thanks to staff of the San Francisco Estuary Institute (Bruce Thompson, Sarah Lowe, and others) for contributions to all aspects of this program, Peggy Myre (Exa Data and Mapping) for data management support, Jay Field (NOAA) for development of the CA LRM, Brian Anderson and other staff of the UC Davis Marine Pollution Studies Laboratory for toxicity method development, and to members of the SQO benthic index development workgroup. Kerry Ritter (SCCWRP) and Bob Smith (deceased) provided much of the statistical analyses for development of the CSI and index interpretation thresholds included in this manual. Chris Beegan (State Water Board) provided essential guidance and input throughout this project as program manager for the Water Board.

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# PREFACE

The incorporation of sediment quality objectives into the State Water Board's water quality policy represents a major development in the application of sediment quality assessment for regulatory programs. Previously, the methods and data interpretation process for sediment monitoring data has been variable due to the lack of a statewide objectives and variable data interpretation methods. The goal of this document is to help organizations make the transition to the new or revised methods specified in the new policy by providing information on methods and data interpretation.

The draft version of this manual is being provided in an effort to assist organizations in developing expertise in the new methods. While much effort has been devoted to checking the information for accuracy, this manual may undergo revision as a result of additional review by other organizations. The reader is encouraged to check the Water Board's sediment quality objectives web page and the SCCWRP sediment quality assessment web pages for future revisions to this document.

This document was prepared the State Water Board's technical team to provide end users with guidance for application of the direct effects tools identified in the State Water Board's Water Quality Control Plan for Enclosed Bays and Estuaries - Part 1 Sediment Quality (referred to as draft SQO policy in this document). This draft document consists of recommended approaches and does not represent regulation or direction from the State Water Board.

# LIST OF ACRONYMS

| | |
|---|---|
| AET | Apparent Effects Threshold |
| ASE | Accelerated Solvent Extraction |
| BPTCP | Bay Protection and Toxic Cleanup Program |
| BRI | Benthic Response Index |
| CA LRM | California Logistic Regression Model |
| CASQO | California Sediment Quality Objectives |
| COC | Chain of Custody |
| CRM | Certified Reference Material |
| CSI | Chemical Score Index |
| DI | De-ionized |
| DQO | Data Quality Objective |
| DNQ | Data Not Quantifiable |
| EC50 | Half Maximal Effective Concentration |
| EMAP | Environmental Monitoring and Assessment Program |
| GC-ECD | Gas Chromatography-Electron Capture Detector |
| GC-MS | Gas Chromatography-Mass Spectrometry |
| GIS | Geographical Information System |
| HPAH | High Molecular Weight Polycyclic Aromatic Hydrocarbon |
| IBI | Index of Biotic Integrity |
| ICP-MS | Inductively Coupled Plasma Mass Spectrometry |
| ICZN | International Commission on Zoological Nomenclature |
| LOE | Line of Evidence |
| LPAH | Low Molecular Weight Polycyclic Aromatic Hydrocarbon |
| MDL | Method Detection Limit |
| MLOE | Multiple Lines of Evidence |
| MSD | Minimum Significant Difference |
| ND | Non-Detect |
| NIT | Negative Indicator Taxa |
| NOAA | National Oceanic and Atmospheric Administration |
| O/E | Observed/Expected |
| PAH | Polycyclic Aromatic Hydrocarbon |
| PCB | Polychlorinated Biphenyl |
| PIT | Positive Indicator Taxa |
| PNA | Percent Normal Alive |
| ppb | parts per billion |
| ppt | parts per thousand |
| RMP | Regional Monitoring Program |
| QA | Quality Assurance |
| QAPP | Quality Assurance Project Plan |
| QA/QC | Quality Assurance/Quality Control |
| QC | Quality Control |
| RBI | Relative Benthic Index |
| RIVPACS | River Invertebrate Prediction and Classification System |

| | |
|---|---|
| RL | Reporting Limit |
| RPD | Relative Percent Difference |
| SAS | Statistical Analysis System |
| SCAMIT | Southern California Association of Marine Invertebrate Taxonomists |
| SCCWRP | Southern California Coastal Water Research Project |
| SWAMP | Surface Water Ambient Monitoring Program |
| SIM | Selected Ion Monitoring |
| SQGs | Sediment Quality Guidelines |
| SQO | Sediment Quality Objective |
| SPMD | Semipermeable Membrane Device |
| SRM | Standard Reference Material |
| SWI | Sediment-Water Interface |
| SWRCB | State Water Resources Control Board |
| TIE | Toxicity Identification Evaluation |
| TOC | Total Organic Carbon |
| TMDL | Total Maximum Daily Load |
| TWV | Taxa Richness Weighted Value |
| USEPA | United States Environmental Protection Agency |

# TABLE OF CONTENTS

# LIST OF FIGURES

# LIST OF TABLES

# CHAPTER 1: INTRODUCTION

## Background

Sediment quality influences the overall condition of a water body. Sediments act as a reservoir for contaminants that can be transferred to the water column and are also a primary source of contaminant exposure for sediment-dwelling organisms. Sediment quality assessment has been an important feature of many California monitoring programs. It was a major focus in the Bay Protection and Toxic Cleanup Program (BPTCP; Anderson *et al.* 1997), the California Environmental Monitoring and Assessment Program (EMAP; USEPA 2005a), the San Francisco Regional Monitoring Program (SFEI 2005), and the Southern California Bight Regional Monitoring Program (SCCWRP 2003, 2007).

Sediment is a complex matrix of components and forms. Consequently, evaluating sediment quality based on a single type of data (line of evidence) is problematic. For example, bulk measures of chemical concentration fail to differentiate between the fraction of a contaminant that is tightly bound to sediment and that which is biologically available. Multiple mechanisms of contaminant exposure, including uptake of chemicals from interstitial water, sediment ingestion, and bioaccumulation through the food web further complicate interpretation of sediment chemistry data. For these reasons, sediment quality assessment often involves simultaneously evaluating multiple lines of evidence (MLOE) that measure both contaminant exposure and effects on organisms: an approach commonly known as the sediment quality triad (Long and Chapman 1985). Lines of evidence (LOEs), such as sediment chemistry, toxicity, and benthic community condition are often used. Virtually all of the ambient sediment quality monitoring programs in this country rely on more than one line of evidence (USEPA 1998, Crane *et al.* 2000, MacDonald and Ingersoll 2002, USEPA 2004). Such programs include the two largest nationwide estuarine monitoring programs: the United States Environmental Protection Agency (USEPA) EMAP and the National Oceanic and Atmospheric Administration (NOAA) National Status and Trends Program, as well as California's BPTCP (Anderson *et al.* 1997, Fairey *et al.* 1998, Phillips *et al.* 1998, Anderson *et al.* 2001, Hunt *et al.* 2001).

In 2003, the California State Water Resources Control Board (SWRCB) initiated a program to develop sediment quality objectives (SQO) for chemical contaminants in bays and estuaries based on an MLOE approach. This first phase of the California SQO (CASQO) program was completed in 2008, which resulted in the SWRCB's adoption of new policy regarding sediment quality as part of the draft water quality control plan for enclosed bays and estuaries (SWRCB 2008). This policy contains two narrative sediment quality objectives: one for the protection of aquatic life due to the direct effects of exposure to sediment contaminants and one for the protection of human health from indirect effects through the consumption of seafood. To implement the direct effects SQO, which is the focus of this document, the policy specifies a series of required analyses and a data interpretation framework based on the integration of three LOEs: 1) sediment chemistry, 2) sediment toxicity, and 3) benthic community (Figure 1.1). While the SQO policy specifies the types of measurements and describes how to interpret the results, many technical details regarding the analysis methods are referenced in other documents. As a result, new users of the CASQO direct effects assessment approach may have difficulty

obtaining the information necessary to apply the tools correctly. The objective of this document is to describe these technical details in an integrated manner in order to facilitate the assessment of sediment quality using the CASQO approach.

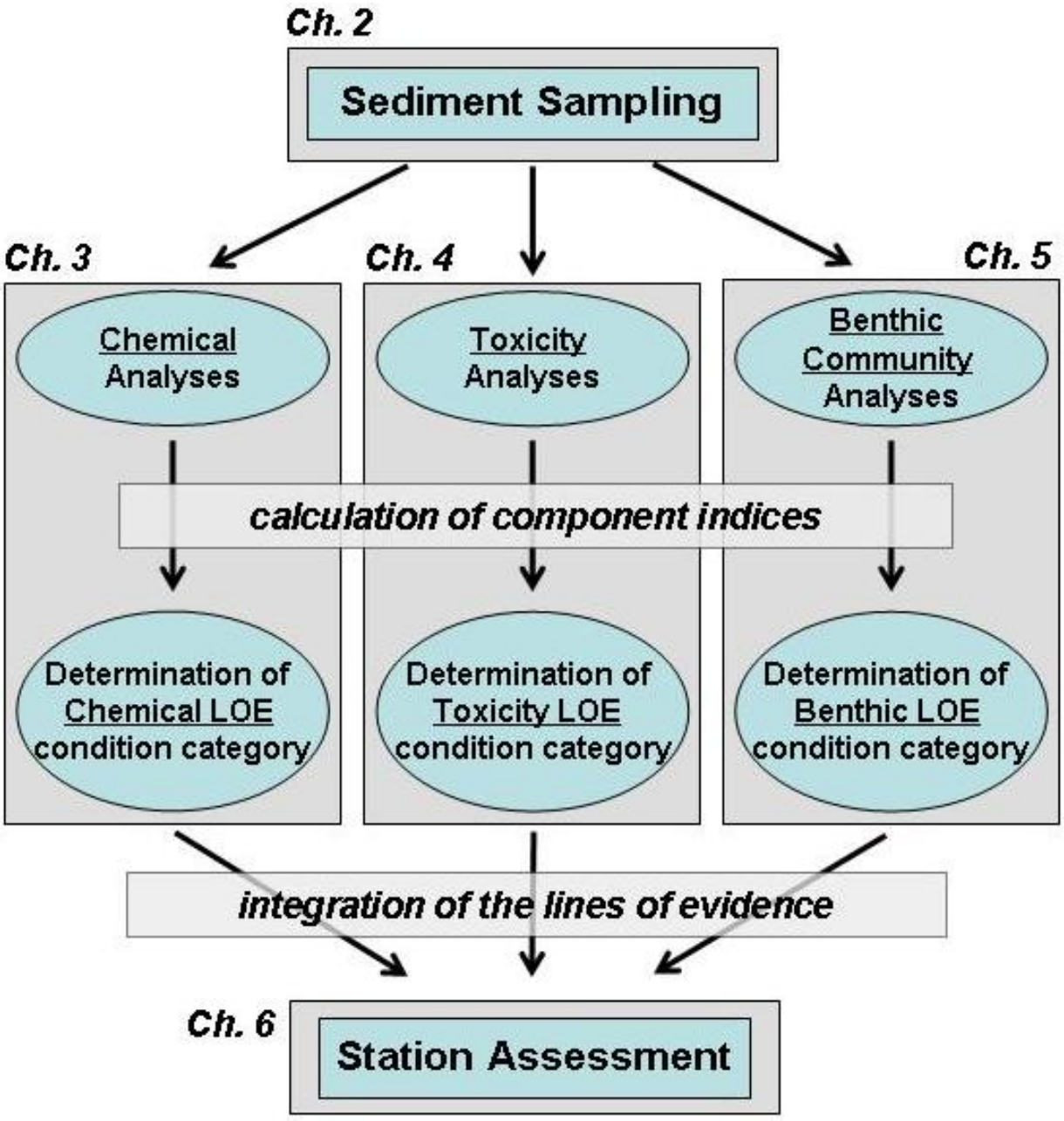


**Figure 1.1. Overview of the station assessment process and chapters addressing each component.**

## Scope of the Manual

This document was prepared the State Water Board's technical team to provide end users with guidance for application of the direct effects tools identified in the State Water Board's Water Quality Control Plan for Enclosed Bays and Estuaries - Part 1 Sediment Quality (referred to as draft SQO policy in this document). This draft document consists of recommended approaches and does not represent regulation or direction from the State Water Board.

This manual describes the methods and provides recommendations for obtaining sediment quality data that is consistent with the draft SQO policy for direct effects. This manual does not provide information for evaluating sediment quality with respect to the SQO for the protection of human health; different measurement and interpretation methods are required for the indirect effects assessment.

The information presented in this document is targeted towards the technician or scientist responsible for generating or analyzing the data, and assumes a basic familiarity with the types of measurements described. It is also intended to serve as a reference for environmental managers in the design and interpretation of monitoring studies.

This manual is intended to supplement current standard methodologies applied in California, rather than providing comprehensive instructions for each type of analysis. As such, the different chapters contain varying levels of detail about sediment assessment procedures based on the amount of information that is already available elsewhere. If methods are published in other documents (as is the case for the sediment chemistry analyses), they are referenced in the text so that the reader may acquire them separately. If no other comprehensive sources for the methods exist (as is the case for some of the methodology described for sediment toxicity and benthic community composition assessment), detailed information is included in this manual. The manual also provides step-by-step instructions and examples for integrating the various data types to result in an assessment of sediment quality that is consistent with the CASQO direct effects assessment framework.

This manual is intended solely to assist end users with making an accurate assessment of sediment quality; it does not provide guidance for how to use the information in a regulatory context. Information on the use of the assessment information in monitoring and regulatory applications is provided in the draft SQO policy and decisions regarding the use of this information are the responsibility of the regulatory and monitoring agencies involved in the program.

## General Considerations for CASQO Assessment

The CASQO assessment approach was developed specifically for application in California enclosed bays and estuaries. While the overall conceptual approach and many of the measurements are appropriate for other habitats, many of the indices and response ranges used to interpret the data have been calibrated to specific habitats and should not be applied to other areas (*e.g.*, offshore waters and freshwater habitats) without additional development and validation.

A variety of environmental conditions exist within bays and estuaries that limit the scope of application of some of the tools, especially benthic community indices. Benthic species composition and abundances vary naturally from habitat to habitat and expectations for reference condition and measurements of deviation from reference should vary accordingly. The benthic indices described in this manual are only applicable for certain euhaline and polyhaline habitats and careful attention should be paid to verify that the appropriate indices are used for the habitat of interest.

Samples are optimally collected during a summer index period from July to September. Physical environments in many enclosed bays are stable and most similar from year to year in the summer. Benthic community composition and abundances have similar stability patterns; measurement of benthic community disturbance is therefore most reliable when sampling is conducted in summer. Sediment samples for each type of analysis (i.e., toxicity, chemistry, benthos) should be collected at the same in order to minimize variability associated with station positioning or seasonal events.

The data integration framework described in this document requires that all three LOEs are measured according to the methods specified. While each LOE provides useful information and can be measured independently, all three LOEs are needed to provide a more accurate and reliable measure of sediment quality.

## Organization of the Manual

This manual is organized into chapters addressing the key components of the CASQO assessment approach (Figure 1.1):

- Chapter 2 provides recommendations for **sediment sampling**
- Chapter 3 provides recommendations for sediment chemistry analysis and shows the steps in deriving the **chemistry line of evidence result (Chemistry LOE)**
- Chapter 4 provides recommendations for sediment toxicity analysis and shows the steps in deriving the **toxicity line of evidence result (Toxicity LOE)**
- Chapter 5 provides recommendations for assessment of the benthic community and shows the steps in deriving the **benthic community line of evidence result (Benthic LOE)**
- Chapter 6 describes how to integrate the three LOEs to generate an overall **station assessment result**

Chapters 3 through 5 each begin with a discussion of general recommendations for sediment sample handling and processing. This is followed by specific procedures and guidance for sample collection, handling, storage, laboratory analyses, quality assurance, data analysis, and interpretation. The chapters also include a sample data set and instructions for using the analytical results to derive the lines of evidence. Chapter 6 describes how to integrate the

information from each of the individual LOEs to derive a single overall sediment condition category. It also discusses interpretation of the condition category and suggestions for next steps depending upon the outcome of the assessment.

# CHAPTER 2: SEDIMENT SAMPLING

## Objectives

Analyses of benthic community condition, chemistry, and toxicity require the collection of samples of surface sediment that are representative of in situ conditions and free from sampling artifacts such as degradation or contamination. Each type of analysis has unique requirements for sample collection, onboard processing, and storage. The objectives of this chapter are to provide an overview of the key elements of field sampling in subtidal marine and estuarine habitats.

## Scope

This chapter is intended to supplement current sediment sampling protocols used in California for enclosed bays and estuaries by providing recommendations for methodology suitable for application within the CASQO assessment framework. It covers a wide range of sampling activities including a discussion of common grab samplers, a summary of sample handling procedures to prepare for eventual laboratory analyses, and a description of recommended approaches for ensuring sample quality and integrity for each type of analysis.

## When and Where to Sample Sediments

Samples are optimally collected during summer months from July to September. Physical environments and benthic community characteristics are relatively stable and most similar from year to year in the summer. This is especially true in areas where rainfall and freshwater influence are high, such as San Francisco Bay and Northern California.

Complete sets of tools for assessing sediment quality in the CASQO program are available only for two of California's six enclosed bay habitats: southern California marine bays and polyhaline central San Francisco Bay. This limitation is primarily based on the lack of a full complement of benthic indices for other embayments. Detailed information on recognizing California benthic habitats is provided in Chapter 5. Benthic species composition and abundances vary naturally from habitat to habitat and expectations for reference condition and measurements of deviation from reference should vary accordingly.

## Sediment Samplers

A wide variety sampling devices are used for collecting sediment. The specific type of sampler used is often determined by the requirements of the monitoring program or habitat characteristics. The primary criterion for selection of a sediment sampler is that repeated sampling consistently collects undisturbed samples of sediments down to at least 5 cm below the sediment surface and that sediment samples, once collected, are not compromised by additional mixing. Any grab sampler to be utilized should meet the following requirements: 1) it is constructed of material that does not introduce contaminants, 2) it creates a minimal bow wave

while descending to the sediment surface to minimize disturbance of the flocculent layer, 3) it takes a sample with minimal disturbance to the sediment surface, 4) it does not leak sample or pore water during retrieval of sample, 5) the sample is easily accessed to verify sample quality and for removal of the sediment surface, and 6) samplers for benthic community condition meet size (surface area) requirements for the geographic area being sampled.

The three major categories of sampling devices for sediment are grabs, corers, and dredges. Dredges are not suitable for sediment quality assessment because they disrupt surface sediments.

### *Grab Samplers*

Grabs are the most frequently used type of sampler for sediment quality assessment. Typically, grab samplers are held open during the descent and are activated upon contact with the bottom. Activation of the sampling jaws can occur by different types of mechanisms: spring tension, pulley arrangement, or lifting wire. The most common benthic sampler is the Van Veen or modified Van Veen grab. Grabs with a surface area of 0.05 $m^2$ are usually used in San Francisco Bay and 0.1 $m^2$ are used elsewhere. Depending on vessel configurations and study needs, smaller surface area grabs may be acceptable.

A Van Veen or equivalent grab is recommended for use in collecting sediment for biology, chemistry, and toxicity analyses. It utilizes a lifting wire to close the jaws of the grab, while the sampler retains its depth of penetration, and is capable of collecting sediment up to a depth of 18 cm. Additionally, Van Veen grabs usually have sampler doors with a mesh covering, which minimizes disturbance of the flocculent layer on sediment surfaces. When the grab is open on descent, the mesh allows water to pass through the sampler, reducing the pressure wave created as the sampler descends to the sediment surface. Another significant advantage of a Van Veen sampler is its large access doors for visual inspection and removal of the upper undisturbed sediment layers. Equivalent grabs with smaller sampling surface area are suitable provided that the sediment sample is equivalent in quality to the Van Veen grab.

### *Core Samplers*

Core samplers represent a diverse category of devices that are usually intended to collect samples in which the sediment stratification is preserved. There are six categories of core samplers: gravity, box, piston, impact, vibrating, and diver-assisted. Of these, only the box and diver-assisted core samplers collect samples compatible with all elements of the sediment quality assessment tools described in this manual. The box corer is used to take undisturbed sediment samples and is especially valuable when deep penetration or a large quantity of sediment is needed. Upon reaching bottom, the weighted box penetrates the surface sediments and a lifting wire activates the lower end of the box, closing it off while the hinged covers seal off the top of the box. Many box corers are large and heavy, which is a significant disadvantage when working off of smaller boats.

The diver-assisted cores consist of glass, metal, or plastic tubes inserted into the sediment and retrieved by a diver. Although the use of divers for core sampling results in high sample placement accuracy and minimal disruption, the sample size is small, resulting in the need to

take a large number of replicate samples from each station in order to provide sufficient material for analysis.

## Sediment Collection

Multiple grab samples are usually required at each station in order to provide sufficient sediment for the assessment framework. There is no required order of sample collection, but the benthic infauna sample is often collected first as this sample is often the most difficult to obtain and may require a longer time for onboard processing than the chemistry and toxicity samples. Because of such challenges, inability to obtain the benthic infauna sample is frequently the reason for failure to successfully sample a location.

### *Grab Evaluation*

Grab sampling might be impossible or very difficult at some sites due to sediment or ocean conditions. Sediment type (percent sand) tends to be a significant determinant of achieving proper penetration depth. Increasing sand content typically decreases penetration depth, such that obtaining a minimum 5-cm penetration depth can be a challenge. Some sediment types (*e.g.,*cobble, gravel, coarse sands) and localities (*e.g.,*canyons, slopes, and rocky areas) could be difficult to sample. Sediments containing rocks and large/intermediate shell debris often prevent complete closure of the grab such that sediment washes out during retrieval.

Each grab sample must be inspected upon retrieval and determined to meet acceptability criteria before it can be used to provide sediment for analyses. The acceptability of a sample must be determined by inspection of the grab contents (Figure 2.1). An acceptable sample condition is characterized by a relatively even surface with minimal disturbance and little or no leakage of the overlying water. Heavily canted samples are unacceptable. Samples with a large amount of "humping" along the midline of the grab, which indicates washing of the sample during retrieval, are also unacceptable. However, some humping will be evident in samples taken from firmer sediment due to the closing action of the grab, such that humping is not necessarily evidence of unacceptable washing.

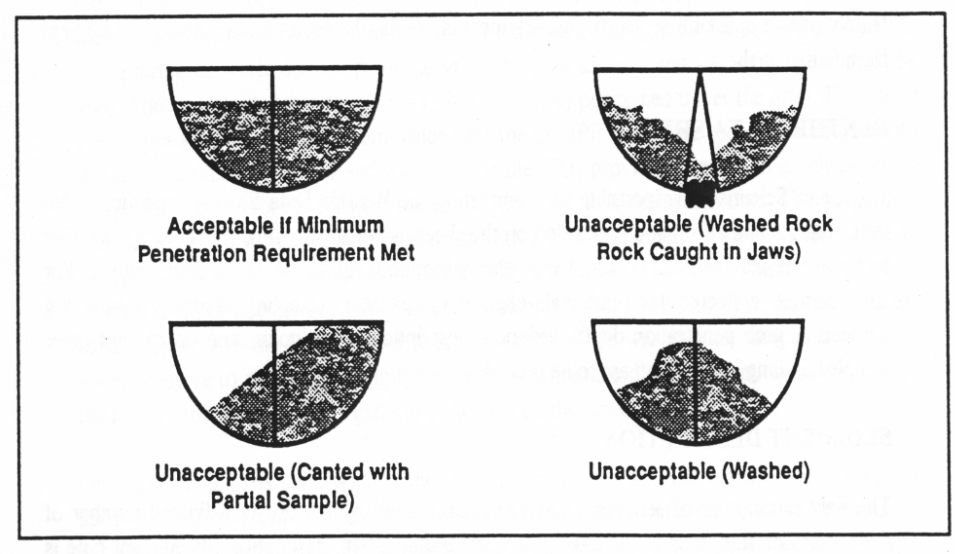


**Figure 2.1. Examples of acceptable and unacceptable grab sample condition (from Tetra Tech, Inc. 1986)**.

Sediment penetration depth must meet the minimum study requirements but should not exceed the capacity of the grab. In habitats where sediments are unusually soft (*e.g.*, some estuary muds), it may be necessary to reduce weight to prevent over-topping the grab due to excessive penetration. All of the grabs taken at a single station should be of similar sediment type. Marked variations in sediment type or grab penetration should be noted and brought to the attention of the field supervisor for a final determination of grab acceptability.

If sample condition is acceptable, the overlying water is drained off (and screened, for benthic community grabs) and the depth of penetration is determined and recorded. Precautions should be taken when draining the overlying water to minimize the loss of surface sediments. It is recommended that a siphon be employed for grabs used for toxicity and chemistry analysis; alternatively the water may be drained off very slowly by slightly opening the jaws of the grab. The overlying water from grabs intended for infaunal analysis should be captured and screened along with the sediment from the grab, as this water may contain benthic macrofauna that are part of the sample.

## Station Occupation and Grab Event Data

### *Station Occupation Data*

Data on the station location and on conditions are recorded for every station. Collection of these data can be best accomplished by using electronic data capture with preformatted station sheets and as much computerized input as possible. This will simplify data recording, promote consistency, minimize errors, and allow for rapid data collection. Computerized input could include automatic recording of GPS coordinates, anemometer readings, and fathometer readings. Manual recording on data sheets is acceptable if a computer system is unavailable or malfunctioning.

Station occupation data usually include:

- Station identification
- Date
- Time of arrival
- Collecting agency identification (or code)
- Vessel name
- System used for navigation
- Weather and sea conditions
- Salinity
- Station fail code identifying reason for abandonment (if site is abandoned)

***Grab Event Data***

All field measurements of sediment characteristics should be made before the sediment is removed from the grab for processing. Information about the grab sample and unusual incidents during sample collection should be documented. Examples of field descriptions and characterization of the sediments are: coarse sand, fine sand, silt or clay, gravel, or a mixed grain size or color. The presence of non-aqueous-phase liquids, such as petroleum tar, and high percentages of shell hash should also be recorded, as should odors, such as hydrogen sulfide (the odor of rotten eggs), petroleum, humic and others, or a lack of noticeable odors. General sediment colors (*i.e.*, black, green, brown, red, olive, or gray) or the presence of a surface sheen should also be recorded. Be aware that sunglasses can interfere with color determinations.

Onboard physical and chemical measurements of sediment parameters may be included, depending on the study design. These are best done before onboard sample processing and immediately, when possible, if there is known instability in the parameter to be measured. Examples of parameters include: sediment pH, redox potential and interstitial or pore water salinity. Sediment that has been disturbed by the measurement activities should not be included in samples for toxicity or chemistry analysis.

Grab event data usually include:

- Time of event (grab on bottom)
- Latitude and Longitude at time of event (grab on bottom)
- Depth of water (where grab on bottom)
- Depth of penetration of grab in sediment (to nearest 0.5 cm)
- Sediment composition (*e.g.*, coarse sand, fine sand, silt or clay, gravel, or mixed grain size)
- Sediment odor
- Sediment color

- Presence of shell hash
- Sample types (*e.g.*, sediment chemistry, sediment toxicity, or benthic community) taken from the grab

## Quality Control Samples

The collection of quality control (QC) samples is recommended, as they facilitate an assessment of the accuracy, precision, representativeness, and bias of the study results. These samples also assess variability in contamination or toxicity associated with sampling procedures. QC samples cost the same to analyze as regular samples, so they should be used judiciously to address important components of the study. Examples of quality control samples are:

- **Field Blank:** Field blanks describe a group of QC samples used to measure sample contamination resulting from field procedures. For example, a travel blank (a type of field blank) consists of a sub-sample of clean sediment (provided by the analytical laboratory) that is transferred from its original container to another clean container during the field sampling procedure. This sediment can be analyzed for all analytes of interest concurrently with the field-collected sediments. An equipment blank (another type of field blank) consists of a clean sample or solvent that is exposed to the sampling device, sample containers, and scoops and then returned to the laboratory for analysis.
- **Field Replicate:** Replicates usually require a separate grab drop at a station. As the name implies these are additional samples taken at the collection site after collecting the original sample. They serve to assess heterogeneity within the station and uniformity in sample handling. Sediment samples are analyzed for the same constituents as the original. Common strategies utilize 5 to 10% replication among study stations. When available, replicate samples provide the ability to conduct statistical analysis between replicates providing a more accurate range of analyte concentrations at the collection site.
- **Duplicate:** Duplicates are split samples of sediment obtained from a single grab or sediment composite sample. Each sample is analyzed separately in order to assess variability associated with the sampling methods. Analytical labs also analyze duplicate samples of sediment from the sample container. The lab duplicate samples are used to assess variability associated with homogenization and analysis of the sample.

## Sample Processing

### *Cleaning Equipment*

There are multiple sources of contamination during sampling, including boat surfaces, vessel exhaust, pelagic species introduction to benthic infauna, sediment carryover, or from skin and clothing of personnel. During sample collection, it is important that any contamination from these sources be minimized. This requires cleaning of all materials in contact with the samples (*i.e.*, grab samplers, mixing bowls, utensils, and storage containers) and screening of intake water.

Proper handling and rinsing of samplers, utensils and mixing bowls are some of the simplest activities that minimize contamination. It is good practice to decontaminate all equipment between sites by washing, rinsing in ambient water, and then rinsing a final time with de-ionized (DI) water (and solvent, if allowed and necessary). Cover and/or store decontaminated gear such as samplers, mixing bowls, and utensils in a clean location between sites. Discard gloves used at the previous site. Put new gloves on upon arrival at a new site.

For multiple grabs within a site, rinsing with ambient water should suffice. The goal is to minimize contamination between grabs, so grab residuals should be discharged in a manner such that they drift away from the sampling spot. Use best judgment to ensure that grab residuals do not contaminate another nearby sampling station. Consult local authorities regarding discharge regulations within harbors and estuaries.

A method proven effective in cleaning equipment between sampling events includes the following steps:

1. Rinse equipment to remove all visible sediment.
2. Scrub all sampling utensils and mixing bowls with a detergent solution, either in a bucket or by using a spray bottle. Also wash all parts of the grab sampler with the detergent solution. Use care, because, depending upon the study and analysis (*e.g.*, endocrine disruption, historical tracers), detergent residue may contaminate the sample and render the results invalid.
3. Completely rinse the grab, buckets, sampling utensils and bowls with raw water making sure they are clear of sediment and detergent residue.
4. Rinse items with 10% HCl followed by a rinse with pesticide-grade methanol. Note: Many vessel captains discourage the use of acid (because it corrodes metal bolts) and solvents (because they dissolve epoxy resins in fiberglass). A containment system should capture all residues (acid/solvent), and a hazardous material container should be used to store the used residue. See Coast Guard regulations regarding handling and storage of hazardous materials onboard a vessel.
5. Completely rinse the grab, buckets, sampling utensils, and bowls with DI water. Note that if insufficient DI water is available, a final rinse with ambient water is acceptable.
6. Cover all cleaned items (except for the grab) with aluminum foil or plastic until the next use in order to minimize exposure to airborne-particle contaminants. Choice of cover material depends upon the study and analytes being measured (*i.e.*, it should not introduce contaminants that could affect the analyses). Rinse all cleaned items with ambient water before use.

### *Processing Benthic Community Samples*

It is recommended that the entire contents of at least one grab at each station be dedicated to analyzing benthic community condition. Complete the grab event data form, wash the sediment

sample from the grab, and then screen, relax, and fix the animals. Recommendations for these procedures are provided below. Note that the water used to wash samples should be filtered to prevent the accidental introduction of surface-water organisms. Be aware that different-sized grabs yield different infauna results.

Screening the Sediment

Typically the grab contents are washed into a tub (≥70 L capacity) positioned under the grab, and the sediment is transferred from the tub into a screening box. Using a sediment-washing table is recommended, but not required. The table provides a flat, smooth surface over which to spread and wash the sample, thus facilitating gentle break up of sediment clumps before they fall off the end of the table into the screening box. The screening box must be equipped with a stainless steel mesh with 1.0-mm openings (or 0.5-mm, for the San Francisco Bay). Wire diameter should be similar to that found in the US Standard 1.0-mm sieve (*i.e.*, 0.0394 inches). The surface area of the screen should be adequate to easily accept the sample without clogging. Typical screen surface areas are 1500 to 2100 $cm^2$.

A smaller screen size or additional screens sizes are used in some studies (*e.g.*, in the San Francisco Bay area, a 0.5-mm screen is utilized to separate smaller organisms from the sediment). These smaller screens are sometimes stacked below the 1.0-mm screen so that the material passes through each screen in sequence and the smaller organisms are captured and separated from the sediment. When all material smaller than 1.0 mm has passed through the top screen, the process is repeated with the finer screen until all material smaller than 0.5 mm has passed through. If the bottom screen (0.5 mm) begins to clog with sediment, the field crew ceases adding sample and gently runs the hose nozzle with low flow along the outside bottom of the 0.5-mm screen being careful not to lose sample by allowing water to escape over the top of the sieve. Note that if the original sample contains many shell fragments and/or worm tubes, the sediment sample should be added to the top (1.0 mm) screen in stages so that the screen does not become too full.

Water pressure must be controlled during washing to avoid damaging the organisms. Direct application of water from a hose without a nozzle to the material and organisms collecting on the screen should be avoided. A fan spray nozzle with a shut-off valve capable of adjusting pressure is typically used.

Note that the necessity of sampling from small craft may prohibit the ability to wash and screen onboard. In these cases, the samples may be screened and processed on land or from the side of the vessel at a temporary screening station established near the sampling location. To assure that the sample does not deteriorate, such off-site screening must be completed as soon as possible and no longer than 90 minutes after sample collection.

Transferring Samples to Containers

Once the sample has been washed through the screen, all the material (debris, coarse sediment, and organisms) retained on the screen is transferred into a sample container. When transferring the material to containers, great care should be taken to avoid damage to the organisms. The sample container should be filled to 50 to 70% of capacity with screened material. After the bulk

of material has been transferred to the container, the screen should be closely examined for any organisms caught in the mesh. These should be carefully removed from the mesh, using pointed forceps or tweezers in order to avoid severing parts, and transferred to the sample container. Between samples, screens should be rinsed with water and scrubbed clean with a stiff-bristle brush. It is important to remove any accumulated debris from screens between samples to minimize the risk of cross-contamination.

A sample may be split between two or more containers if it is too large for one. Label the sample container with an external label containing the sampling agency name, station name, sample type, date, and container number (*i.e.*, 1 of 1, 2 of 3, etc.). An internal label bearing the same information should be placed inside each infauna sample container. This label should be written in pencil or indelible ink on 100% rag paper, poly-paper, or other paper of a quality suitable for wet labels. The sample container must have a screw-cap closure and be sufficiently large to accommodate the sample material with a head-space of at least 30% of the container volume. To facilitate this, field crews should have a wide range of sample container sizes available to them.

Relaxing and Fixing the Specimens

All infaunal samples should be treated with a relaxant solution for approximately 30 minutes prior to fixation in 10% buffered formalin. Either an Epsom salts ($MgSO_4$) solution or a propylene phenoxytol solution (formulations below) may be used as a relaxant. Relaxant solutions may be used as the diluent water for the fixative, or may be decanted off after relaxation is complete and replaced with diluted fixative. If it is used as diluent water, fill the sample container to 85 to 90% of its volume, close the container and gently invert it several times to distribute the solution. Leave the sample in the relaxant for 30 minutes. After 30 minutes, top off the container with enough sodium borate buffered formaldehyde to achieve a 10% formalin solution. Close the container, and gently invert it several times to assure mixing. Store the sample for return to the laboratory.

If the relaxant solution is not used as the diluent water, the relaxant must be removed from the sample container and replaced with 10% buffered formalin. After the 30 minutes of relaxation, decant the relaxant from the sample through a screen of the mesh size used previously to screen the sample. Remove all organisms from the screen and transfer them to the sample container. Fill the container with sodium-borate-buffered 10% formalin (rather than the undiluted buffered formaldehyde). Close the container, gently invert it several times, and store it for return to the laboratory. Samples should be kept in fixative for no less than 72 hours. However, within two weeks of collection, they should be returned to the laboratory, washed, and transferred to a 70% ethanol preservative (see Chapter 5 for details). Thereafter samples can be held at relatively constant temperatures (<30°C), out of sunlight, for a year before the preservative must be refreshed.

Relaxant and fixative stock solution recipes:

| | |
|---|---|
| **Epsom salts relaxant solution**: | 1.5 kg Epsom salts ($MgSO_4$ @ $7H_2O$) per 20 L of freshwater |
| **Propylene phenoxytol solution**: | 30 ml propylene phenoxytol to 20 L of seawater |
| **Buffered formalin solution**: | 50 g sodium borate ($Na_2B_4O_7$) per liter of formalin |
| **Buffered 10% formalin solution**: | 1 part buffered formalin to 9 parts fresh or saltwater |

***Processing Surface Sediment Samples for Chemistry and Toxicity Analysis***

All of the toxicity and chemistry analyses described in this manual are conducted on the surface sediment collected from the upper 5 cm of the grab/core sample. In contrast to the benthic infauna sample, the chemistry and toxicity samples are usually obtained from the same grab samples in order to maximize the comparability of the data. Up to 4 liters of sediment may be required for all of the analyses, with the bulk of the sediment used for toxicity testing. As a result, multiple sediment grabs are almost always required to obtain sufficient sediment for analysis.

With the exception of samples for sediment-water interface toxicity tests (see following), the surface sediment is removed from the sampler using a non-contaminating scoop that is usually specially fabricated to remove only the desired depth of sediment. One popular scoop design resembles a metal box with the top and one end removed. The sides of the box are 5 cm high in order to provide a depth reference, a metal handle is attached and the entire assembly is coated with a non-contaminating material.

Two strategies are typically used for sample processing and allocation of sub-samples: composited or noncomposited. In the noncomposited strategy, separate samples for chemistry and toxicity are obtained from the same (or subsequent) grabs and they are placed in containers specific for the analysis type. An effort is usually made to obtain comparable and representative samples for each type of analysis by obtaining sediment from multiple locations within each grab and using sediment from multiple grabs for each type of analysis. This subsampling strategy minimizes the potential for chemical contamination of the samples and provides the greatest flexibility in terms of materials used for sediment scoops and sample containers.

The composite sample processing strategy is similar to that described above, except that the sediment is placed into an intermediate mixing container and homogenized prior to filling the storage jars. From a toxicological perspective, it is preferable that the sediment be composited and homogenized prior to distribution into the sample containers. This method maximizes

comparability of the chemistry and toxicity samples and may provide more options for the location of the sample allocation step (*e.g.*, homogenization and filling of sample jars can take place within a laboratory area of the ship, which may be a cleaner environment). Both the mixing container and utensil (*e.g.*, spoon, spatula) used for taking the sub-sample should be of non-contaminating material. An inert coating, such as Teflon, would be acceptable for the mixing bowl and utensils.

Unless the grab sampler is coated with a non-contaminating material, sediment in contact with, or within 1 cm of, the metal sides should be avoided to prevent sample contamination. Furthermore, to prevent contamination during collection of sub-samples, all containers, scoops, and related gear should be covered when not in use.

***Processing Sediment Samples for Sediment-Water Interface Toxicity Tests***

The Sediment-Water Interface (SWI) test is used to assess toxicity of solid phase sediment samples using the embryo or larval stages of marine and estuarine invertebrates. This test is designed to be conducted on a relatively undisturbed core sample containing the upper 5 cm of sediment, which requires the use of the special sample processing methods described below. If the study design calls for the SWI test to be conducted on homogenized samples, then the sample processing methods described in the previous subsection should be employed.

Intact sediment can be taken from grab sampler or directly from the bottom by a diver. Sediment is collected from a grab sample with a polycarbonate core (7.5 cm id). This sub-sample must be the first sediment taken from an undisturbed grab. The core is pressed 5 cm into the sediment and a pre-cleaned acrylic plate or a gloved hand is inserted under the bottom of the core to prevent loss of sample as the core is removed. It is convenient to mark the height (5 cm) for reference around the outside of the core. After the core is removed from the grab, it is gently wiped of exterior sediment and the bottom is capped quickly with a polyethylene plastic cap (7.5 cm id); the top is then capped.

Alternatively, SWI test sediment cores can be collected directly from the bottom by diver-assisted coring, especially in shallow, soft-bottom sediments. The core is pressed 5 cm, or other prescribed depth, into the bottom sediment, and a pre-cleaned acrylic plate or a gloved hand is inserted under the bottom of the core to prevent leakage of sample or interstitial water as the sub-sample is removed. The bottom and top of the core are capped by a second diver while at the bottom. The sample is then brought to the boat or land staging area.

Core sub-sample integrity is verified by the presence of sediment overlying water and the required depth of sediment. If an inordinate volume of sediment is lost, the sample is discarded and a new one collected. A small hole in the top cap relieves positive pressure on the sample and minimizes leakage as the cap is attached. Once capped, the outside of the core is washed, and the core is placed upright in a cooler for storage and transport. Care must be taken to minimize tilting, shaking or vibrating these cores during transport. Precautions should also be taken to prevent contamination of the core contents by water from melting ice during storage.

## Sample Storage

Recommended conditions for sample handling and storage are listed in Table 2.1. Additional detailed analysis-specific recommendations are presented below the table.

**Table 2.1. Recommended sample sizes, containers, preservation techniques, and storage times for sediment.**

| Material | Minimum Sample Volume ($ml^3$)[a] | Container Type[b] | Preservation Technique | | Storage Time (months)[c] |
|---|---|---|---|---|---|
| | | | Transport | Storage | |
| Grain Size | 70 | HDPE or Glass | Wet ice then 4°C | | 6 |
| Total Organic Carbon | 135 | HDPE or Glass | Wet ice then 4°C | -20°C | 6 |
| Metals | 70 | HDPE or Glass | Wet ice then 4°C | -20°C | 12 |
| Total Mercury | 35 | Glass | Wet ice then 4°C | -20°C | 6 |
| Organics | 135 | Glass | Wet ice then 4°C | -20°C | 12 |
| Chemistry Archive | 200 | Glass | Wet ice then 4°C | -20°C | |
| Toxicity Tests | 3000 | HDPE or Glass | Wet ice then 4°C | 4°C | 1 |
| Acute Amphipod Exposure | | | | | |
| *10-day Whole Sediment Test* | 1500 | | | | |
| Acute Mussel Embryo Exposure | | | | | |
| *2-day Sediment-Water Interface Test* | 1500 | | | | |
| Chronic Polychaete Exposure | | | | | |
| *28-day Whole Sediment Test* | 1000 | | | | |

[a] Minimal volume to conduct analyses or a single toxicity test with appropriate controls.

[b] Recommended container, but other types are suitable.

[c] Recommended storage times used by multiple programs.

- **Sediment Grain Size:** This sample should be placed in a glass or high-density polyethylene (HDPE) container, or a Whirlpak®, taking care to leave an air space at the top. Samples should be stored at > 0 to 4°C by placing them on wet ice or in a refrigerator until submitted to the laboratory. **Do not freeze these samples.**

- **Total Organic Carbon:** This sample should be placed in a glass or HDPE container with a Teflon-lined lid. The container should be 75 to 80% full, taking care to leave an air space at the top to prevent breakage of the container due to expansion of the sample during freezing. Samples should be stored at > 0 to 4°C by placing them on wet ice or in a refrigerator, and must be frozen within 24 hours.

- **Metals:** This sample should be placed in an acid-cleaned glass or HDPE container with a Teflon-lined lid. This container should be 75 to 80% full, leaving an air space at the top to prevent breakage of the container due to expansion of the sample during freezing.

Samples should be stored at > 0 to 4°C by placing them on wet ice or in a refrigerator, and must be frozen within 24 hours.

- **Organics:** This sample should be placed in a solvent-rinsed (or pre-certified clean) glass container with a Teflon-lined lid. The container should be 75 to 80% full, taking care to leave an air space at the top to prevent breakage of the container due to expansion of the sample during freezing. Samples should be stored at > 0 to 4°C by placing them on wet ice or in a refrigerator, and must be frozen within 24 hours.

- **Chemistry Archive:** This sample should be placed in a solvent-rinsed (or pre-certified clean) glass container, with a Teflon-lined lid. The container should be 75 to 80% full, taking care to leave an air space at the top to prevent breakage of the container due to expansion of the sample during freezing. Samples should be stored at > 0 to 4°C by placing them on wet ice or in a refrigerator, and must be frozen within 24 hours.

- **Toxicity:** This sample should be maintained in a glass or a HDPE plastic container with a Teflon-lined lid, taking care to leave an air space at the top. Samples should be stored in the dark at > 0 to 4°C by placing them on wet ice or in a refrigerator until returned to the laboratory. **Do not freeze these samples.** Samples should be analyzed within four weeks of sampling.

- **Toxicity - *Sediment-Water Interface Test*:** This sample should be maintained intact in its core and sealed at the bottom to prevent leakage. The core should remain upright, in order to not disturb stratification and be maintained in the dark at > 0 to 4°C. If the cores are cooled by ice, precautions should be taken to prevent contamination of the cores by melting ice. **Do not freeze these samples.** Samples should be analyzed within a week of sampling if possible, with a maximum storage time of 4 weeks.

# CHAPTER 3: SEDIMENT CHEMISTRY

Sediment chemistry is an essential line of evidence (LOE) required for sediment quality assessment. The Chemistry LOE, which is the California Sediment Quality Objectives (CASQO) chemistry endpoint, helps determine the type of chemical exposure and its potential for producing adverse biological effects. Determination of the Chemistry LOE is comprised of two main components: 1) measurement of a suite of constituents and 2) interpretation of the results using indices of chemical exposure that are based on sediment quality guidelines (SQGs).

This chapter provides computational tools for determining the Chemistry LOE category. The data analysis procedure described includes calculation of chemical contamination indices based on two types of SQGs: 1) the California Logistic Regression Model (CA LRM) and the 2) Chemical Score Index (CSI). Integration of these two indices yields the Chemistry LOE. At the end of the chapter, an example of the step-by-step process for determining the Chemistry LOE category is provided.

## Objectives

The objective of this chapter is to describe the sediment-chemistry analyses needed to apply the CASQO framework. The information in this chapter is intended to supplement laboratory protocols commonly used for monitoring California's subtidal marine and estuarine habitats by indicating those constituents and methods needed to obtain data consistent with the requirements of the CASQO framework.

## Scope

The methods described in this chapter focus only on the sediment constituents that must be assessed in order to conduct the CASQO station assessment. The lack of description of specific contaminants or methods is not intended to imply that they are not important in other elements of a sediment quality assessment program. As with any program, the specific study design and project objectives should determine what is measured.

## Sediment Chemistry Constituents

In order to generate the Chemistry LOE, a specific set of sediment chemistry constituents should be measured. These are provided in Table 3.1. The recommended maximum reporting limits (RLs) listed for each constituent are based on the CSI classification ranges and do not necessarily reflect the maximum performance achievable with available analytical methods. The concentrations associated with each RL are expressed on a dry-weight basis.

Table 3.1 should not be interpreted as an exhaustive list of all analytes that might be of interest in a sediment quality assessment study. Each program will need to determine what other analytes (*e.g.*, certain pesticides) might be of value, depending on the objectives of the monitoring program. In addition, it should be noted that some other analytes that are not required by the

Chemistry LOE should also be measured in order to generate the Toxicity and Benthic Community LOEs. These include total organic carbon (TOC) and percent fines.

**Table 3.1. Constituents to be analyzed for sediment chemistry determination within the CASQO framework and their corresponding recommended maximum reporting limits (RLs).**

| Target Analytes | Maximum RLs |
|---|---|
| **Metals:** | |
| Cadmium (mg/kg) | 0.09 |
| Copper (mg/kg) | 52.8 |
| Lead (mg/kg) | 26.4 |
| Mercury (mg/kg) | 0.09 |
| Zinc (mg/kg) | 112 |
| **Polycyclic Aromatic Hydrocarbons (PAHs):** | |
| *Low Molecular Weight PAHs:* | |
| Acenaphthene (μg/kg) | 20.0 |
| Anthracene (μg/kg) | 20.0 |
| Phenanthrene (μg/kg) | 20.0 |
| Biphenyl (μg/kg) | 20.0 |
| Naphthalene (μg/kg) | 20.0 |
| 2,6-dimethylnaphthalene (μg/kg) | 20.0 |
| Fluorene (μg/kg) | 20.0 |
| 1-methylnaphthalene (μg/kg) | 20.0 |
| 2-methylnaphthalene (μg/kg) | 20.0 |
| 1-methylphenanthrene (μg/kg) | 20.0 |
| *High Molecular Weight PAHs:* | |
| Benzo(a)anthracene (μg/kg) | 80.0 |
| Benzo(a)pyrene (μg/kg) | 80.0 |
| Benzo(e)pyrene (μg/kg) | 80.0 |
| Chrysene (μg/kg) | 80.0 |
| Dibenz(a,h)anthracene (μg/kg) | 80.0 |
| Fluoranthene (μg/kg) | 80.0 |
| Perylene (μg/kg) | 80.0 |
| Pyrene (μg/kg) | 80.0 |
| **Organochlorine Pesticides:** | |
| Alpha Chlordane (μg/kg) | 0.50 |
| Gamma Chlordane (μg/kg) | 0.54 |
| Trans Nonachlor (μg/kg) | 4.6 |
| Dieldrin (μg/kg) | 2.7 |
| o,p'-DDE (μg/kg) | 0.50 |
| p,p'-DDE (μg/kg) | 0.50 |
| o,p'-DDD (μg/kg) | 0.50 |
| p,p'-DDD (μg/kg) | 0.50 |
| o,p'-DDT (μg/kg) | 0.50 |
| p,p'-DDT (μg/kg) | 0.50 |

**Table 3.1 Continued.**

| Target Analytes (Continued) | Maximum RLs (Continued) |
|---|---|
| **Polychlorinated Biphenyls (PCB congener numbers):** | |
| 2,4'-Dichlorobiphenyl (μg/kg) (8) | 3.0 |
| 2,2',5-Trichlorobiphenyl (μg/kg) (18) | 3.0 |
| 2,4,4'-Trichlorobiphenyl (μg/kg) (28) | 3.0 |
| 2,2',3,5'-Tetrachlorobiphenyl (μg/kg) (44) | 3.0 |
| 2,2',5,5'-Tetrachlorobiphenyl (μg/kg) (52) | 3.0 |
| 2,3',4,4'-Tetrachlorobiphenyl (μg/kg) (66) | 3.0 |
| 2,2',4,5,5'-Pentachlorobiphenyl (μg/kg) (101) | 3.0 |
| 2,3,3',4,4'-Pentachlorobiphenyl (μg/kg) (105) | 3.0 |
| 2,3,3',4',6-Pentachlorobiphenyl (μg/kg) (110) | 3.0 |
| 2,3',4,4',5-Pentachlorobiphenyl (μg/kg) (118) | 3.0 |
| 2,2',3,3',4,4'-Hexachlorobiphenyl (μg/kg) (128) | 3.0 |
| 2,2',3,4,4',5'-Hexachlorobiphenyl (μg/kg) (138) | 3.0 |
| 2,2',4,4',5,5'-Hexachlorobiphenyl (μg/kg) (153) | 3.0 |
| 2,2',3,4,4',5,5'-Heptachlorobiphenyl (μg/kg) (180) | 3.0 |
| 2,2',3,4',5,5',6-Heptachlorobiphenyl (μg/kg) (187) | 3.0 |
| 2,2',3,3',4,4',5,6-Octachlorobiphenyl (μg/kg) (195) | 3.0 |

## Sediment Chemistry Methodology

Recommendations for sample preparation, extraction/clean-up, and analysis for each of the CASQO sediment chemistry constituents are provided in Table 3.2

The use of USEPA-approved methods such as the "*Test Methods for Evaluating Solid Waste, Physical/Chemical Methods*" (also known as SW-846; USEPA 2005c) is recommended. If standard methods are not available, approval of alternative methods should first be obtained from the State Water Resources Control Board. Additional methods may be acceptable if they produce results that are at or below the desired reporting limits and are comparable to results that would be generated by EPA SW-846.

**Table 3.2. Commonly used and recommended (USEPA SW-846[1]) extraction, clean-up, and determinative methods for sediment chemistry analysis.**

| Analyte | Extraction/Digestion | Clean-up | Determinative |
|---|---|---|---|
| Sediment Grain Size | | | Percent Solids EPA 160.3 |
| Total Organic Carbon | | | Carbonaceous Analyzer EPA 9060, 5310 |
| Metals | | | |
| Cd, Cu, Pb, Zn | Nitric/Hydrochloric Acid Digestion EPA 3050 | | Flame Atomic Absorption (FLAA) EPA 7000 |
| | | | Graphite Furnace Atomic Absorption (GFAA) EPA 7010 |
| | | | Inductively Coupled Argon Plasma-Optical Emission Spectometry (ICP-OES) EPA 6010 |
| | | | Inductively Coupled Argon Plasma-Mass Spectometry (ICP-MS) EPA 6020 |
| Hg | Aqua Regia Digestion EPA 7471 | | Cold Vapor Atomic Absorption (CVAA) EPA 7471 |
| | Acid Digestion EPA 7474 | | Cold Vapor Atomic Fluorescence (CVAF) EPA 7474 |
| Organics | Soxhlet Extraction EPA 3540 | Sulfur Removal EPA 3660 | |
| | Sonication Extraction EPA 3550 | Gel-Permeation Chromatography EPA 3640 | |
| | Microwave Assisted Extraction EPA 3051 | Alumina EPA 3600, 3610 | |
| | Pressurized Fluid Extraction EPA 3545 | Florisil EPA 3620 | |
| PAHs | | | GC-MS (in SIM mode) EPA 8121 |
| | | | Flame Ionization Detection EPA 8015 |
| Pesticides | | | GC-MS EPA 8270 |
| | | | GC-ECD EPA 8081 |
| PCBs | | Strong Acid EPA 3565 | GC-MS 8081 |
| | | | GC-MS (in SIM mode) EPA 8082 |

[1]Method reference refers to the latest promulgated revision of the method, even though the method number does not include the appropriate letter suffix.

## Sample Handling, Preservation, and Storage

Since the majority of analyses require trace-level detection limits (*i.e.*, parts per billion; ppb), mitigation of contamination sources is paramount. Because of the challenges associated with trace-level measurements, it is recommended that the laboratory conducting these analyses have experience in quantifying these constituents at comparable RLs (see Table 3.1) on a routine basis

Caution should be taken to avoid contamination at each stage of sample collection, handling, storage, preparation, and analysis. All sample containers should be purchased pre-cleaned or be pre-washed in accordance with methods described below or comparable methods. Sample containers and labware should be cleaned and stored according to sample type and analyte of concern. Handling or touching of insides of glassware should be avoided, as gloves or utensils can introduce residues such as plasticizers to the samples.

Proper rinsing of containers is necessary to eliminate soap residues that can interfere with analysis of certain analytes. It is recommended that Teflon-coated squirt bottles be employed to hold solvents or acids used for rinsing sample containers and labware. Labware that does not appear clean or that is etched should be removed from trace analysis work. With the exception of volumetric labware, high temperature glass labware for trace organics analysis work should be baked at 500°C to remove contaminant residues.

When samples are received by the laboratory, sample acceptance requirements specified in the project Quality Assurance Project Plan (QAPP) should be adhered to in order to ensure sample integrity. Chain of Custody (COC) procedures should be conducted by personnel who are properly trained and authorized to handle incoming sample bottles and records. The following should be verified:

- Sample identification (*i.e.*, these should be congruent between the sample container and the field sheet)
- Acceptable condition of sample bottles (*i.e.*, none should be broken or improperly capped)
- Sample receipt within holding time (refer to Table 2.1)
- Appropriate sample preservation and storage to ensure stability of the analyte

When applicable, any safety hazards associated with the samples should also be noted and documented, and the appropriate personnel should be notified.

Sediment samples should be stored in the dark at $4^{o}$C, on ice, or frozen at $-20^{o}$C, as required for the analytes prior to extraction. Due to the unstable nature of some of the analytes of interest, it is suggested that holding times be as short as possible (see Table 2.1 for example) and that extracts are analyzed as recommended in SW-846. In all cases, an analysis must start prior to expiration of the holding time.

## Sample Preparation and Analysis

Multiple analyses are often conducted on different sediment aliquots. Therefore, it is important to ensure that sediment is well homogenized before aliquotting samples (*e.g.*, for metals or for organics analyses). This includes re-incorporating any overlying water into the sample before taking an aliquot.

Recommended determinative methods for the CASQO target analytes are listed in Table 3.2. The analytical methods selected should be ones that are routinely conducted by the contracted laboratories. The laboratories should be familiar not only with the methods, but also with the guidelines and quality controls necessary for the analytes in question. Also, as a general recommendation, the analytical method chosen for a given analyte should be one that is capable of achieving the target RL or lower.

### *Sediment Grain Size*

Grain size is the measure of particle size distribution for sediments. This is most commonly reported as percent fines (silt + clay) and percent coarse grain (sand). However, distributions are also measured and reported in *phi* size categories, which can be translated into fines and coarse-grain groups.

The primary consideration in conducting grain-size analysis is the need to use standardized sieve mesh sizes for fractioning the size classes of the sediment to be quantified. To ensure comparability of data among laboratories, the following methodology is recommended. Grain size distribution should be measured by either laser- and light-scattering procedures or by pipette analysis. Regardless of which procedure is chosen, gravel should first be separated from finer particles using a size 2000-µm mesh sieve (and then quantified), and the pass-through sediment should then be separated from finer particles using size 1000-µm mesh (and then quantified). For the former procedure, the material that passes through this second sieve is then subjected to laser/light-scattering assessment of the distribution of the remaining size particles. If the pipette method is chosen, a third screening of sediment should be done with size 630-µm mesh to isolate the fines. The pass-through material is then subjected to pipette analysis of size-particle distribution.

## Analyte-Specific Recommendations

### *Total Organic Carbon Preparation and Analysis*

To prepare samples for TOC analysis, frozen sediments are thawed to room temperature and homogenized before being dried in an air oven at 60$^{o}$C overnight. The dried samples are exposed to concentrated hydrochloric acid vapors in a closed container to remove the inorganic carbon. TOC samples can be analyzed by various methods that include high temperature combustion and UV/persulfate oxidation. Analytical grade acetanilide (99.95+ %) is recommended as an external standard for TOC. A certified reference material, such as the PACS-1 marine sediment (National Research Council of Canada), is recommended for evaluating analytical performance.

### *Metals Sample Preparation*

Samples for metals may be prepared for analysis either as wet sediment, dried at room temperature, oven dried, or freeze-dried. If room temperature or oven drying is used, care should be taken in the drying process to minimize volatilization of analytes in the samples by not exceeding a temperature of 60$^{o}$C. To avoid potential problems, analysis of wet or freeze-dried samples is preferred. Sediment metals results must be reported per dry weight. In order to do this, a separate aliquot from the original sample is taken and dried to determine the moisture content.

Sample Digestion for All Metals Except Mercury

The recommended digestion method for target metals other than mercury is the strong acid digestion (EPA Method 3050 (USEPA 2005c) or EPA 1638 Modified). The alternative

procedure, total acid digestion, is not recommended because it uses hydrofluoric and perchloric acids and can result in safety hazards. It can also result in high dissolved solids that cause physical and spectral interferences for all the determinative methods. Such interferences can be severe enough to require dilution of the digestate, resulting in higher reporting limits. Furthermore, the development of the CASQO chemical indices was based on analyses of metals data obtained using strong acid digestion. As such, use of a method other than strong acid digestion may affect the accuracy of the Chemistry LOE determination.

Sample Digestion and Analysis for Total Mercury

EPA Methods 7471 and 245.5 (USEPA 1991, 2005c) can be used for sediment digestion for mercury analysis. These methods use *aqua regia*, a mixture of one part concentrated nitric acid and three parts concentrated hydrochloric acid, as part of the digestion process and result in quantitative recoveries for total mercury in marine sediments. The use of *aqua regia* rather than the nitric/sulfuric acid mix specified in EPA Method 245.1 (USEPA 1994) is effective for highly organic sediment samples.

### *Metals Analysis*

A variety of methods are available to quantify metals in the samples (Table 3.2). However, most monitoring programs use inductively coupled plasma mass spectrometry (ICPMS). Cold Vapor Atomic Absorption or Cold Vapor Atomic Fluorescence EPA 7471 (USEPA 2005c) are the recommended techniques for analysis of mercury in marine sediments.

### *Organics Preparation*

Clean-up procedures are usually necessary before the analysis of organic compounds from extracts of marine sediments to maximize accuracy and precision of results. Methods for clean-up are detailed in Table 3.2 and include sulfur removal, chromatography, and the use of strong acid/oxidizers.

Sample preparation for organics analyses generally involves extraction from the sample matrix followed by isolation and concentration of target analytes prior to instrumental analysis. Common extraction procedures for organic contaminants in marine sediments are taken from USEPA SW-846 (Table 3.2), including: Soxhlet Extraction, Sonication Extraction, Microwave Assisted Extraction, and Pressurized Fluid Extraction (also called Accelerated Solvent Extraction (ASE; EPA 3545; USEPA 2005c). ASE has shown promise to maintain or improve on extraction efficiencies while greatly reducing solvent volumes and extraction times. It should be noted that modifications to any of the methods listed may be necessary to achieve low-level detection limits. Modifications can include reducing the final volumes cited by the method, starting with larger sample sizes, or both.

As with metals, organic results must be reported on a dry-weight basis. Therefore a separate sub-sample or aliquot from the original homogenized sediment sample should be analyzed for moisture content.

### *Organics Analysis*

Polychlorinated Biphenyl (PCB) congeners and organochlorine pesticides are usually analyzed by either dual-column gas chromatography electron capture detection (GC-ECD; EPA 8081) or gas chromatography mass spectrometry (GC-MS; EPA8270) in the selected ion monitoring (SIM) mode (EPA 8082). Polycyclic Aromatic Hydrocarbons (PAHs) are generally analyzed by GC-MS or flame ionization detection (EPA 8015).

## Quality Assurance/Quality Control

The following section provides recommendations on the Quality Assurance/Quality Control (QA/QC) elements that should be included in a sediment chemistry assessment program in order to generate high quality data. In addition to confirming data quality, these elements can also provide insight into problems with the data so that appropriate corrective actions can be taken.

From the standpoint of analytical methodology, it should be noted that a significant portion of what is recommended in this chapter is based on "*Test Methods for Evaluating Solid Waste, Physical/Chemical Methods*," SW-846 (USEPA 2005). While modifications of standard analytical procedures may also be acceptable for application to the CASQO framework, any such methods should be demonstrated to provide results with levels of precision and accuracy that equal, or exceed, those generated through the standard protocols. As such, when modified methods for sediment chemistry analysis are employed, the use of "performance-based methodology" is strongly encouraged in order to achieve a level of data quality consistent with the CASQO Program. This approach typically involves development of Data Quality Objectives (DQOs; see below) that are relevant for, and compatible with, the desired use in terms of timeliness, completeness, accuracy, and precision.

### *Analysis Sensitivity*

Sediment chemistry analyses for application within the CASQO framework must be conducted with a degree of sensitivity sufficient to generate a meaningful Chemistry LOE. For each analyte, it is necessary to identify the minimum level at which there is high technical confidence in the quantified result (*i.e.*, a threshold above which there is a low probability of either a false positive or false negative). This is accomplished by defining a RL for each analyte. The RL is the minimum concentration that can be measured by a given lab, using a given methodology, without risking substantial interferences. By definition, any result above the RL can be reported in the project database without any sort of qualification stating that it is an *estimated* quantity.

The RL is related to another measure of sensitivity, the Method Detection Limit (MDL), which can be determined empirically based on the standard deviation of low-level matrix spike responses. The MDL indicates the level of "noise" inherent in the analytical methodology used by a given laboratory. Any results falling below the MDL cannot be distinguished from zero and therefore should be qualified as such, with no reporting of numerical values (see below). Knowledge of the MDL achievable by a given laboratory is crucial to understanding whether sufficiently low concentrations of the analyte can be reliably quantified for ultimately deriving the Chemistry LOE.

While RLs are often around 5 to 10 times higher than corresponding MDL for a given analyte, the establishment of RLs is ultimately at the discretion of the laboratory conducting the analyses.

Recommended Reporting and Detection Limits

For use of sediment chemistry results within the CASQO framework, it is recommended that the RLs for all the target sediment chemistry constituents be on par with those presented in Table 3.1. Laboratories should report the RL and the MDL for each analysis. This information should also be included in the project dataset. The MDL is important to include because it facilitates Chemistry LOE calculations in situations in which target analyte results fall below reporting limits (see below).

Result Qualifier Codes

Results reported at the RL or above correspond to lower uncertainty and thus are believed to be more accurate than those below the RL. However, detectable levels of target constituents between the RL and MDL can also provide some valuable information. Thus, it is recommended that data be reported as follows:

- if $x \geq RL$, report the determined concentration; no data qualification is necessary
- if $MDL \leq x < RL$, report the estimated concentration; also add a data-qualifier that indicates a lower level of confidence in the result, such as *data not quantifiable* (DNQ)
- if $x < MDL$, do not report a value; report *non-detect* (ND) in the qualifier section

***Data Quality Objectives***

It is very important to establish the validity of the sediment chemistry data prior to using them to generate the Chemistry LOE. A robust dataset should include a full suite of QA/QC samples that are indicative of how successfully sampling and laboratory analytical procedures were carried out. DQOs should also be set for each of the QA/QC sample types. Examples of acceptable DQOs that have been employed in marine sediment chemistry studies are provided in Table 3.3.

The output for the QA/QC samples in each analytical run should be compared to the pre-established DQOs as a measure of the reliability of that run's results. In addition to this, as a matter of course, all laboratories should keep detailed notes during sample preparation and analysis. All of this information will be used to validate the data and troubleshoot any problems.

For each sample batch (traditionally defined as a group of up to 20 samples), it is recommended that at least one each of the following QA/QC sample types be included in the analytical run:

- A *blank* to determine the likelihood that samples in the batch have been contaminated
- A *matrix spike* to evaluate the potential for interference(s) between component of the sample matrix and the analysis of the target constituent
- A *duplicate* to estimate the precision of the results, by calculating the relative percent difference (RPD)

- A *standard* using certified reference material (CRM) to assess the accuracy of the analytical procedure

**Table 3.3. Example of Data Quality Objectives (DQOs) for sediment chemistry analysis (adapted from the Bight '03 QAPP). RPD = Relative Percent Difference.**

| QA/QC Sample Type | Data Quality Objective |
|---|---|
| **Blanks** | |
| Frequency | 1/batch |
| Accuracy | < MDL |
| **Certified Reference Material (CRM)** | |
| Frequency | 1/batch |
| Accuracy | Within ±30% of certified value for 80% of analytes |
| **Matrix Spikes** | |
| Frequency | 1/batch |
| Accuracy | Within ±30% of true value |
| Precision | RPD <30% |
| **Sample Duplicates** | |
| Frequency | 1/batch |
| Precision | RPD <30% |

### *Data Validation*

Before using any analytical results in calculations to derive the Chemistry LOE, each data report should be carefully inspected in order to determine the validity of the analytical run and the completeness of data reporting. Recommended data validation measures include confirming the following:

- Reporting units and numbers of significant figures are correct
- MDLs and RLs have been reported by the laboratory for each analyte and are within the recommended limits provided in Table 3.1
- Initial and continuing instrument procedural blank levels are consistent with laboratory QA/QC guidelines
- Initial and continuing calibration of laboratory instrumentation meets laboratory QA/QC guidelines
- QA/QC samples (blanks, duplicates, matrix spikes, percent recovery surrogates, and CRM/Standard Reference Material (SRM)) have met or exceeded DQOs
- Reported concentrations for each analyte fall within "environmentally-realistic" ranges, as deduced from previous studies and expert judgment

Corrective Actions

If the datavalidation process reveals a problem, this information in combination with the review of laboratory comments can help to identify and rectify it. Data that are deemed suspect because of failure to meet DQOs should be re-evaluated and flagged with data qualifiers, where appropriate. Depending on the severity of the problem, re-sampling and re-analysis of some or all samples may be necessary. Any corrective actions should be taken before subsequent sample batches are analyzed, and technical interpretation/reporting and use of the data should begin only after the full QA/QC review has been completed.

## Data Management and Reporting

Once data quality has been deemed satisfactory, all raw data (including the result of all quality-assurance samples) should be entered in a project database whose format is standardized and therefore accessible to other parties. A report summarizing the process and outcome of data evaluation should also be prepared to accompany the database.

Sediment chemistry data should be stored in a database using a standard format that will be accessible to other users. Several possible formats are available, and some examples include the databases for the State Water Board's Surface Water Ambient Monitoring Program (SWAMP), the CASQO program, and the Southern California Bight Regional Monitoring Surveys. A report summarizing the QA/QC review of the data package should be prepared and made available to potential users of the database. Laboratory data and accompanying explanatory narratives should also be archived.

Reports documenting the results of the QA/QC review of a data package should summarize all conclusions concerning data acceptability and note significant problems. These reports are useful in providing data users with a written record of any data concerns and a documented rationale for why certain qualified data were either accepted as estimates or rejected.

The following items should be addressed in a QA/QC report:

- Summary of overall data quality, including a description of data that were qualified
- Brief descriptions of analytical methods and the method(s) used to determine MDLs
- Description of data reporting, including any corrections made for transcription or other reporting errors, and description of data completeness relative to objectives (*e.g.,* 90% complete) stated in the QAPP
- Descriptions of initial and ongoing calibration results, blank contamination, and precision and bias relative to QAPP objectives and stated DQOs (including tabulated summary results for CRMs, laboratory control materials, and matrix spikes/matrix spike duplicates)

## Calculation of the Chemistry LOE

The Chemistry LOE is based on a combination of two sediment chemistry indices that determine the magnitude of chemical exposure at a site. The chemistry indices are based upon two types of sediment quality guideline approaches: 1) a logistic regression model calibrated to California data (CA LRM; Bay *et al*. 2008) and 2) the Chemical Score Index (CSI; Ritter *et al*. 2008).

The CA LRM was developed using an EPA logistic regression modeling approach that estimates the probability of toxicity based on the chemical concentration (Field *et al*. 2002, USEPA 2005b). The CSI uses chemistry data to predict the occurrence and severity of benthic community disturbance. Index-specific response ranges are applied to each index to classify the result into one of four chemical exposure categories: Minimal, Low, Moderate, and High. The resulting exposure categories are assigned a score of 1 to 4 (*e.g.*, Minimal Exposure = 1) and the average of the scores for each chemistry index is used to determine the overall Chemistry LOE category.

The specific chemical constituents used in the indices were selected as part of the CASQO tool-development process and are listed in Table 3.4. Note that each index uses a subset of the constituents. Selection of these constituents was based on multiple factors including data availability and index performance. It should be noted that omission of other contaminants from the list in Table 3.4 does not imply that such contaminants are not potentially important factors influencing sediment quality.

Because the CA LRM and CSI indices are based on data for specific constituents, substitutions or omissions thereof may result in an inaccurate determination of the Chemistry LOE, and are therefore not recommended. If for some reason it is necessary to omit one or more of these constituents, this information should be reported to the study manager and used to qualify the CASQO results accordingly.

### *Chemical Category Sums*

Six of the constituents in Table 3.4 represent the sum of multiple chemicals (*i.e.,* low molecular weight PAH (LPAH), high molecular weight PAH (HPAH), total PCBs, total DDTs, total DDEs, and total DDDs). The specific compounds comprising the sums of the PAH and PCB groups are listed in Table 3.1. Total DDTs represents the sum of p,p'-DDT and o,p'-DDT; total DDEs represents the sum of p,p'-DDE and o,p'-DDE; total DDDs represents the sum of p,p'-DDD, and o,p'-DDD. The compounds making up each group were based on those used in the National Oceanic and Atmospheric Administration (NOAA) Status and Trends program.

**Table 3.4. CASQO sediment chemistry target constituents, the Chemistry LOE indices for which they are used, and example values used for the demonstration calculations in this chapter.**

| Sediment Constituent | Applicable Index/Indices | | Example Concentration |
|---|---|---|---|
| | CSI | CA LRM | |
| Cadmium (mg/kg) | | X | 0.15 |
| Copper (mg/kg) | X | X | 43.6 |
| Lead (mg/kg) | X | X | 33.5 |
| Mercury (mg/kg) | X | X | 1.37 |
| Zinc (mg/kg) | X | X | 45.4 |
| HPAH (µg/kg) | X | X | 1672 |
| LPAH (µg/kg) | X | X | 261 |
| Alpha Chlordane (µg/kg) | X | X | 3.1 |
| Gamma Chlordane (µg/kg) | X | | 2.4 |
| Dieldrin (µg/kg) | | X | 1.7 |
| Trans Nonachlor (µg/kg) | | X | 2.5 |
| DDDs, total (µg/kg) | X | | 6.7 |
| DDEs, total (µg/kg) | X | | 2.7 |
| DDTs, total (µg/kg) | X | | 10.6 |
| 4,4'-DDT (µg/kg) | | X | 2.5 |
| PCBs, total (µg/kg) | X | X | 22.7 |

The sums for HPAH, LPAH, DDTs, DDDs, and DDEs are calculated by adding the reported (*i.e.*, quantified) value of each individual compound, expressed on a dry weight basis. Compounds qualified as non-detected are treated as having a concentration of zero for the purpose of summing. If all components of a sum are non-detected, then the highest reporting limit of any one compound in the group should be used to represent the sum value.

A slightly different summation method is used for the PCBs in order to compensate for the use of a shorter list of PCB congeners than the NOAA program. The concentrations for the individual PCB congeners are summed as describe above. This total PCB sum is then multiplied by a correction factor of 1.72 to approximate the value obtained using the larger NOAA list. A reduced list of congeners was selected for the CASQO program in order to provide greater compatibility with California historical data sets, which often have a reduced congener list.

## Example of Chemistry LOE Calculation

This section demonstrates the process for data preparation and calculation to generate the Chemistry LOE for the CASQO assessment framework. The data used in this demonstration are shown in Table 3.4. They represent all the sediment chemistry constituents that are recommended for inclusion within the CASQO framework. The sample data provided are within

ranges that are typical for each constituent for the sediment of California marine and estuarine habitats.

All of the necessary calculations can be carried out using a standard desk calculator or a spreadsheet program, such as Microsoft Excel. For convenience, the Southern California Coastal Water Research Project (SCCWRP) website provides a spreadsheet tool for these calculations. Note that this spreadsheet tool is periodically updated to incorporate input from users; the current version can be found on the Sediment Quality Assessment Tools page of the SCCWRP web site (http://www.sccwrp.org/view.php?id=565).

Data Preparation

The first step in the Chemistry LOE calculations is to confirm that the data are in the proper format. All constituents must be expressed on a sediment dry-weight basis. Specifically, all metals should be in mg/dry kg and all organic constituents should be in µg/dry kg. Note that if calculations using non-detected (ND) analytes are necessary, an estimated value must be used. One estimation approach is to use 50% of the MDL for any samples with ND results for that analyte; however, the previous section should be consulted for addressing ND values within summed groups of constituents.

Calculation of Component Indices

To generate the Chemistry LOE score, the values of the CA LRM and the CSI must first be calculated. Those values are then integrated into a single Chemistry LOE category value for each sampling location. It should be noted that the CA LRM and the CSI indices do not utilize all the same sediment chemistry constituents. While cadmium, dieldrin, trans nonachlor and 4,4'-DDT are solely utilized in the CA LRM calculation, gamma chlordane, total DDDs, total DDEs and total DDTs are solely utilized in the CSI calculation. All other target constituents are used in both indices. The first two columns of Table 3.4 indicate which of the indices utilizes each of the constituents.

### *California Logistic Regression Model*

The CA LRM uses a logistic regression model to predict the probability of sediment toxicity based on sediment chemical constituent concentrations. The relationships between concentration and probability of toxicity have been established for all of the constituents used in the CA LRM (Bay *et al*. 2008). An example, for cadmium, is shown in Figure 3.1.

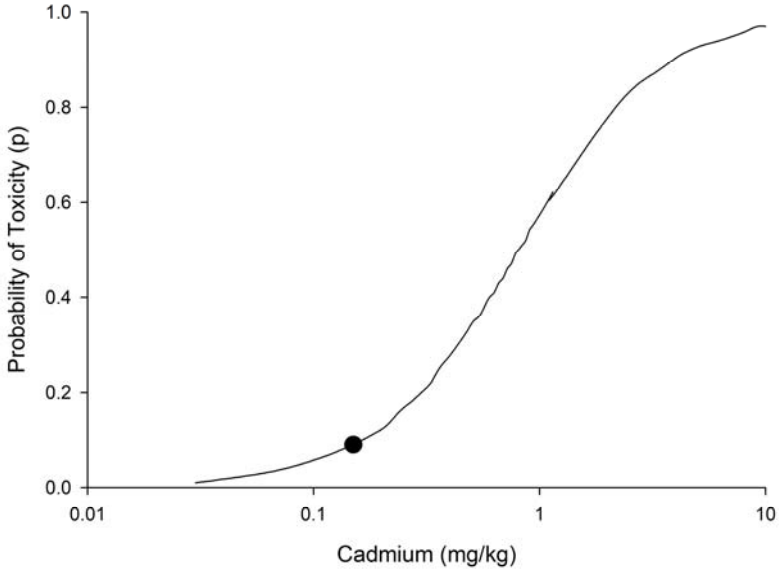


**Figure 3.1. Logistic regression curve relating sediment cadmium concentration to probability of toxicity. The solid circle indicates the calculated probability of toxicity (>0.1) based on a cadmium concentration of 0.15 mg/kg.**

In order to determine the probability of toxicity for all of the target constituents, the concentration data for each is entered in the following logistic regression equation:

$$p = e^{B0+B1\,(x)} / (1 + e^{B0+B1\,(x)})$$

Where: p = the probability of observing a toxic effect;

B0 = the intercept parameter (a *constant*, provided in Table 3.5);

B1 = the slope parameter (a *constant,* provided in Table 3.5); and,

x = the log of the concentration of the analyte of interest (a *variable,* user-entered).

The result of each calculation is rounded to two decimal places.

Table 3.5 provides the values of B0 and B1 that should be used for the various sediment chemistry constituents to determine the CA LRM. It also shows the p values calculated for each target analyte given the data in Table 3.4.

**Table 3.5. CA LRM parameters (constants B0 and B1) and p results (calculated) based on the data in Table 3.4.**

| Chemical | B0 | B1 | p value |
|---|---|---|---|
| Cadmium | 0.2894 | 3.1764 | 0.09 |
| Copper | -5.5931 | 2.5885 | 0.21 |
| Lead | -4.7228 | 2.8404 | 0.40 |
| Mercury | -0.0618 | 2.6837 | 0.58 |
| Zinc | -5.1337 | 2.4205 | 0.25 |
| HPAH | -8.1922 | 1.9995 | 0.15 |
| LPAH | -6.8071 | 1.8827 | 0.09 |
| Alpha Chlordane | -3.4080 | 4.4570 | 0.23 |
| Dieldrin | -1.8344 | 2.5890 | 0.22 |
| Trans Nonachlor | -4.2590 | 5.3135 | 0.10 |
| PCBs, total | -4.4144 | 1.4837 | 0.08 |
| 4,4'-DDT | -3.5531 | 3.2621 | 0.09 |

Using the same logistic regression equation, the probability (p) of cadmium toxicity, based on data from Table 3.4 and parameters from Table 3.5, would be determined as follows:

$$p = e^{0.2894 + 3.1764 * \log(0.15)} / (1 + e^{0.2894 + 3.1764 * \log(0.15)})$$

$$p = e^{-2.328}/(1 + e^{-2.328})$$

$$p = 0.09749/1.09749$$

**p = 0.09** (indicated by the dot in Figure 3.1)

The maximum p value among the target analytes from a given sediment sample is referred to as the "Pmax" value for that sample. The Pmax for the results in Table 3.5 corresponds to mercury. This Pmax value of **0.58** is compared to a set of response ranges to determine the CA LRM category for the sample. Table 3.6 provides these categories. A Pmax value of 0.58 places the sample in the Moderate Exposure category (>0.49 to 0.66≤), which yields a category score of **3**. Thus 3 is the CA LRM result for the site in the example.

**Table 3.6. Response ranges of Pmax for determination of the CA LRM category score.**

| Category | Range | Category Score |
|---|---|---|
| Minimal Exposure | <0.33 | 1 |
| Low Exposure | ≥0.33 - 0.49≤ | 2 |
| Moderate Exposure | >0.49 - 0.66≤ | 3 |
| High Exposure | >0.66 | 4 |

### *Chemical Score Index*

The CSI is calculated independently of the CA LRM, and requires a four-step process. The first step involves comparing the concentration of each chemical constituent (*e.g.*, the data in Table 3.4) to a series of concentration ranges that correspond to predicted benthic disturbance level (Ritter *et al.* 2008). Where the chemical constituent falls within these ranges determines the benthic disturbance category score (Table 3.7).

**Table 3.7. Chemical concentration ranges for the predicted benthic disturbance categories used in the CSI calculation.**

| Chemical Constituent (mg/kg dry weight) | Benthic Disturbance Category | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Copper | ≤52.8 | >52.8 - ≤96.5 | >96.5 - ≤406 | >406 |
| Lead | ≤26.4 | >26.4 - ≤60.8 | >60.8 - ≤154 | >154 |
| Mercury | ≤0.09 | >0.09 - ≤0.45 | >0.45 - ≤2.18 | >2.18 |
| Zinc | ≤112 | >112 - ≤200 | >200 - ≤629 | >629 |
| HPAH | ≤312 | >312 - ≤1325 | >1325 - ≤9320 | >9320 |
| LPAH | ≤85.4 | >85.4 - ≤312 | >312 - ≤2471 | >2471 |
| Alpha Chlordane | ≤0.50 | >0.50 - ≤1.23 | >1.23 - ≤11.1 | >11.1 |
| Gamma Chlordane | ≤0.54 | >0.54 - ≤1.45 | >1.45 - ≤14.5 | >14.5 |
| DDDs, total | ≤0.50 | >0.50 - ≤2.69 | >2.69 - ≤117 | >117 |
| DDEs, total | ≤0.50 | >0.50 - ≤4.15 | >4.15 - ≤154 | >154 |
| DDTs, total | ≤0.50 | >0.50 - ≤1.52 | >1.52 - ≤89.3 | >89.3 |
| PCBs, total | ≤11.9 | >11.9 - ≤24.7 | >24.7 - ≤288 | >288 |

In the second step, the CSI for each constituent is calculated by multiplying its benthic disturbance category score by a chemical weight, provided in Table 3.8.

$$CSI = \Sigma(w_i * cat_i)/\Sigma w$$

Where: $cat_i$ = predicted benthic disturbance category for chemical i (from Table 3.7);

$w_i$ = weight factor for chemical i (from 3rd column in Table 3.8); and

$\Sigma w$ = sum of all weights.

**Table 3.8. Results of CSI calculations based on example dataset in Table 3.4.**

| Chemical | Category (*determined* from Table 3.7) | Weight (a *constant*) | CSI (*calculated*) |
|---|---|---|---|
| Copper | 1 | 100 | 100 |
| Lead | 2 | 88 | 176 |
| Mercury | 3 | 30 | 90 |
| Zinc | 1 | 98 | 98 |
| HPAH | 3 | 16 | 48 |
| LPAH | 2 | 5 | 10 |
| Alpha Chlordane | 3 | 55 | 165 |
| Gamma Chlordane | 3 | 58 | 174 |
| DDDs, total | 3 | 46 | 138 |
| DDEs, total | 2 | 31 | 62 |
| DDTs, total | 3 | 16 | 48 |
| PCBs, total | 2 | 55 | 110 |
| Sum | | 598 | 1219 |
| Weighted Mean (CSI Sum/weight Sum) | | **2.04** | |

The third step is to sum the CSI values for all target analytes and divide by the sum of all the weights (shown on the bottom of Table 3.8). If data are missing for any constituent, both the CSI value and weight for that constituent become zero, thus adjusting both the sum of the CSIs and sum of all weights accordingly.

The final part of the process is to compare the weighted mean CSI value to a series of ranges to determine the CSI category. These ranges are provided in Table 3.9. The CSI value for the example data in Table 3.8 is **2.04**, which places it in the low exposure category from Table 3.9, yielding a category score of **2**. Thus 2 is the CSI result for the site in the example.

**Table 3.9. Response ranges for CSI calculation.**

| Category | Range | Category Score |
|---|---|---|
| Minimal Exposure | <1.69 | 1 |
| Low Exposure | ≥1.69 - 2.33≤ | 2 |
| Moderate Exposure | >2.33 - 2.99≤ | 3 |
| High Exposure | >2.99 | 4 |

Integration of the Sediment Chemistry Indices

The final step in calculating the Chemistry LOE is to integrate the results for the two sediment chemistry indices: CA LRM and CSI. This is achieved by calculating the average of their two category scores. If the average falls between two response ranges, the value is rounded up to the next integer. The rounding methodology was specified by the SWRCB in order to provide a conservative estimate of the Chemistry LOE when the index results disagree. The numeric average can be also expressed as a descriptive category corresponding to the score. For the example data, the category score for the CA LRM was 3 and the Category Score for the CSI was 2. The average is 2.5, which rounds up to 3, yielding a Chemistry LOE category of Moderate Exposure.

# CHAPTER 4: SEDIMENT TOXICITY

Sediment toxicity provides two types of information in this assessment: 1) the potential bioavailability of contaminants and 2) a measure of contaminant biological effects. Multiple toxicity tests are needed to assess toxicity because no single method exists that can capture the full spectrum of potential contaminant effects. Toxicity assessment under the CASQO framework requires information from two types of tests: 1) short-term amphipod survival and 2) a sub-lethal test.

## Objectives

This chapter provides a description of the sediment toxicity test methods specified under the draft SQO policy. The document is intended to supplement published toxicity protocols by providing information on specific aspects of the methods that are used in many California monitoring programs so that future analyses will yield comparable and high-quality results. This chapter also provides instructions for interpreting toxicity data relative to the California Sediment Quality Objectives (CASQO) assessment framework.

## Scope

The sediment toxicity methods described in this manual are based on an evaluation of methods conducted by Bay *et al.* (2007). These toxicity methods include both standardized tests of amphipod survival and sublethal tests using polychaete (*Neanthes arenaceodentata*) growth in sediment and mussel (*Mytilus galloprovincialis*) embryo development at the sediment-water interface (SWI). Many other types of sediment toxicity tests are used to assess sediment quality (*e.g.*, pore water and elutriate tests), but they are not included in this manual because they have not been specified for use in the CASQO program. While the CASQO program is limited to application in bays and estuaries, the toxicity methods described here are also appropriate for assessing sediment toxicity in other habitats (*e.g.*, offshore waters), as long as the exposure conditions are within the tolerance range of the species.

## General Study Considerations

### *Selection of Test Species*

The various species used in toxicity tests often have different tolerances to sediment physical characteristics (*e.g.*, grain size), sensitivities to contaminants, and associations between response and sediment exposure. There is no single "perfect" toxicity test method or species that can measure all aspects of sediment toxicity that are important for sediment quality assessment. Consequently, a suite of multiple toxicity methods is needed to provide a complete assessment of sediment toxicity. At a minimum, this suite should include at least one short-term survival test and one sublethal test.

The sediment toxicity methods described in this chapter are summarized in Table 4.1. A variety of other methods are used in other programs to assess toxicity, but only those specified in the CASQO program are described here. The draft SQO policy requires the use of at least one acute test using amphipods and one sublethal test from Table 4.1. Use of alternate methods may be valuable as a supplement to those in Table 4.1, but they are not used in the determination of the Toxicity line of evidence (LOE).

**Table 4.1. Sediment toxicity test methods recommended for use in California marine habitats.**

| Test Type / Species | Taxonomic Group | Matrix | Duration (days) | Endpoint(s) |
|---|---|---|---|---|
| Acute | | | | |
| *Eohaustorius estuarius* | Amphipod | Whole Sediment | 10 | Survival |
| *Leptocheirus plumulosus* | Amphipod | Whole Sediment | 10 | Survival |
| *Rhepoxynius abronius* | Amphipod | Whole Sediment | 10 | Survival |
| Sublethal | | | | |
| *Neanthes arenaceodentata* | Polychaete | Whole sediment | 28 | Growth, Survival |
| *Mytilus galloprovincialis* | Mussel | Sediment-water Interface | 2 | Embryo Development |

The tolerance of the test species to the characteristics of the test sample should be considered when selecting the test methods for an individual study. For example, extremes in sediment particle size may influence the survival response of an amphipod, which may confound interpretation of the results. Potential confounding factors include, but are not limited to, grain size, total organic carbon (TOC) content, ammonia, and salinity. Where known, information regarding sensitivity to confounding factors is presented in tables within the description of each method.

Another important factor to consider when choosing test methods is the suspected toxicants of concern at a location. For example, *Eohaustorius estuarius* has a relatively high tolerance to copper and may not be a sensitive measure of sediment toxicity where copper is the primary toxicant (McPherson and Chapman 2000). Therefore, it would be a poor choice for testing under these conditions. Conversely, amphipods are very sensitive to organophosphorus and pyrethroid pesticides.

Finally, choice of test method may be dictated by project-specific objectives or conditions. If historical data are available from a site, it may be best to use the same test species that was previously used in order to make temporal comparisons for trends analyses. For regional monitoring programs, use of a consistent suite of tests is helpful in increasing data comparability among surveys or regions. The test method selection may also be helpful in investigating the results of prior studies. For example, if benthic community data indicate that a particular

taxonomic group of organisms is impacted at a site, then using a member of that taxon in a toxicity test may be helpful in understanding the cause of the impact.

### *Sample Preparation*

Chapter 2 provides information on field collection methods and sample storage. Unlike chemistry and benthic infauna samples, toxicity samples cannot be stored for extended periods. The toxicity tests should be started within one month of sample collection in order to minimize potential changes in toxicity due to storage. Samples should be tested as soon after collection as possible in order to minimize the potential for changes in sediment quality during storage.

Sediment for all methods except the SWI test should be press sieved in order to remove native animals that might be either predators or be the same species as a test organism. Press sieving consists of forcing the sediment through a 2-mm mesh screen without adding water beyond that which was already associated with the sample naturally. Press sieving is not necessary for the SWI test because the test organisms are enclosed within a screened chamber that prevents the entry of predators.

The sediment sample should be homogenized in the laboratory prior to addition to the test chambers. Regardless of whether the sample was originally homogenized at the time of collection, it should be homogenized in the laboratory to ensure that each replicate test chamber contains a representative sample. Sediment cores used for the SWI test are not homogenized. As a result, the SWI test results may show greater variability between replicates as a result of small-scale variation in sediment characteristics. This increased variation is a consequence of the test design and is not an indication of poor quality technique. The test response values used to interpret the results take this variation into account.

### *Animal Acclimation*

All of the test species in Table 4.1 are available from commercial vendors who either collect them from the field or raise them in culture facilities. Availability of test animals from commercial sources is never guaranteed and should be confirmed in the planning stages of a study. The test animals used in each method must be acclimated (*i.e.*, with respect to temperature and salinity) to test conditions within each laboratory prior to the start of testing. The acclimation period required for each species is variable. The duration for each species can be found in a table within each method description below. The amphipod *Leptocheirus plumulosus* and the polychaete *N. arenaceodentata* can be cultured and laboratories may choose to use animals from in-house cultures rather than from commercial sources.

### *Interpretation of Test Results*

Interpretation of the test results for use within the CASQO assessment framework requires the test response to be classified into one of four categories:

- Nontoxic: Response not substantially different from that expected in sediments that are uncontaminated and have optimum characteristics for the test species (*e.g.*, control sediments).
- Low Toxicity: A response that is of relatively low magnitude; the response may not be greater than test variability.
- Moderate Toxicity: High confidence that a statistically significant toxic effect is present.
- High Toxicity: High confidence that a toxic effect is present and the magnitude of response includes the strongest effects observed for the test.

The test response category is determined by comparing the results to a set of response ranges that are specific to the test species (Table 4.2). Classification of the test response requires four types of summary data for each test: 1) mean control response, 2) mean response for test sample, 3) test sample response expressed as a percentage of the control, and 4) determination of statistical significance of result from control. Once the toxicity test data are properly formatted, results for each individual toxicity test are simply compared to the ranges for the response categories shown in Table 4.2. Additional instructions for analyzing the test response data are presented as flow charts in the method descriptions for each test species and in the example at the end of this chapter.

**Table 4.2. Sediment toxicity response classification ranges.**

| Test Species/Endpoint | Nontoxic (Percent) | Low Toxicity (Percent of Control) | Moderate Toxicity (Percent of Control) | High Toxicity (Percent of Control) |
|---|---|---|---|---|
| *Eohaustorius* Survival | 90 to 100 | 82 to 89[a] | 59 to 81[b] | < 59 |
| *Leptocheirus* Survival | 90 to 100 | 78 to 89[a] | 56 to 77[b] | < 56 |
| *Rhepoxynius* Survival | 90 to 100 | 83 to 89[a] | 70 to 82[b] | < 70 |
| *Neanthes* Growth | 90 to 100[c] | 68 to 90[a] | 46 to 67[b] | < 46 |
| *Mytilus* Normal Development | 80 to 100 | 77 to 79[a] | 42 to 76[b] | < 42 |

[a] If the response is not significantly different from the negative control, then the response is classified as Nontoxic.
[b] If the response is not significantly different from the negative control, then the response is classified as Low Toxicity.
[c] Expressed as percentage of control.

The toxicity response classification ranges were established for each test organism by analyses of data for California samples using the methods described in Bay *et al.* (2007). The ranges are based on the following criteria:

- The range representing the Nontoxic category is equivalent to the control acceptability criterion for the test method
- The lower bound of the Low Toxicity category range is based on the 90$^{th}$ percentile Minimum Significant Difference (MSD) that is specific to each test species
- The lower bound of the Moderate Toxicity category range is based on the mean of two values:
    - the 99$^{th}$ percentile MSD value
    - the test response corresponding to the 75$^{th}$ percentile of toxic samples

## Acute Test Methods

### *Amphipod 10-day Survival*

All three acute methods use species of amphipods in 10-day whole sediment exposures. Two of these species, *Eohaustorius estuarius* and *Rhepoxynius abronius,* have been used in numerous monitoring and assessment studies in California (Fairey *et al.* 1998; Bay *et al.* 2000, 2005). Tests using *Leptocheirus plumulosus* have been used infrequently in California, but have been widely used in monitoring and assessment studies on the East and Gulf coasts (McGee *et al.* 1999, Lewis *et al.* 2006) and are required for testing drilling muds (Federal Register, January 22, 2001). All three species are burrowers: *E. estuarius* and *R. abronius* burrow freely, and *L. plumulosus* lives in U-shaped burrows.

*E. estuarius* and *R. abronius* are collected from the field by commercial vendors for use in toxicity testing. As such, conditions of temperature and salinity are variable at the collection sites. *L. plumulosus* can either be cultured in the laboratory or collected in the field, but has the same temperature and salinity requirements for acclimation, whether coming from culture or field conditions. It is important that the animals be brought slowly to test conditions before acclimation begins. Temperature must not be adjusted more that 3°C per day and salinity not more than 5 g/kg per day.

Test Method

The methodology for all three methods can be found in USEPA (1994) and ASTM (1996). Test parameters for each method can be found in Table 4.3. Each method is conducted in 1-L chambers containing 2 cm of test sediment and approximately 800 ml of overlying water. As shown in Table 4.3, standard test salinity and temperature vary between methods, but for all methods, samples should be gently aerated, and tests should be conducted under constant light. At least five replicates of each treatment must be tested.

To prepare for tests, the test chambers should be set up with sediment, water, and aeration the day before animals are added. Twenty amphipods in the 2- to 5-mm range are then added to

each replicate chamber. Food is not provided during testing for any of the methods, and there is no renewal of overlying water during the exposure period.

**Table 4.3. Test characteristics for 10-day acute amphipod exposures using *Eohaustorius estuarius*, *Leptocheirus plumulosus* and *Rhepoxynius abronius*.**

| Parameter | *E. estuarius* | *L. plumulosus* | *R. abronius* |
|---|---|---|---|
| 1. Temperature | 15 ±1°C | 25 ±1°C | 15 ±1°C |
| 2. Salinity | 20 ±2 g/kg | 20 ±2 g/kg | 28 ±2 g/kg |
| 3. Illuminance | 500 - 1000 lux | 500 - 1000 lux | 500 - 1000 lux |
| 4. Photoperiod | Continuous light | Continuous light | Continuous light |
| 5. Acclimation | 2 - 10 days at test temperature and salinity | 2 - 10 days at test temperature and salinity | 2 - 10 days at test temperature and salinity |
| 6. Size and life stage | 3 - 5 mm | 2 - 4 mm no mature animals | 3 - 5 mm |
| 7. Number of organisms/chamber | 20 | 20 | 20 |
| 8. Number of replicates/treatment | 5 | 5 | 5 |
| 9. Aeration | Enough to maintain 90% saturation | Enough to maintain 90% saturation | Enough to maintain 90% saturation |
| 10. Water quality measurements | Temperature daily. pH, salinity, ammonia and DO of overlying water at $T_0$ and $T_{final}$. Pore water pH, salinity, ammonia at $T_0$ and $T_{final}$. | Temperature daily. pH, salinity, ammonia and DO of overlying water at $T_0$ and $T_{final}$. Pore water pH, salinity, ammonia at $T_0$ and $T_{final}$. | Temperature daily. pH, salinity, ammonia and DO of overlying water at $T_0$ and $T_{final}$. Pore water pH, salinity, ammonia at $T_0$ and $T_{final}$. |
| 11. Feeding | None | None | None |
| 12. Test acceptability criteria | Mean control survival of ≥90 and ≥80% survival in each replicate. | Mean control survival of ≥90 and ≥80% survival in each replicate. | Mean control survival of ≥90 and ≥80% survival in each replicate. |
| 13. Grain size tolerance | 0.6 - 100% sand | 0 - 100% sand | 10 - 100% sand |
| 14. Ammonia tolerance | <60 (total, mg/L) | <60 (total, mg/L) | <30 (total, mg/L) |
| 15. Total sulfide tolerance | 1.9 (mg/L) | Not Available | 1.5 (mg/L) |

Quality Assurance

A 10-day, water-only reference toxicant test using ammonia should be performed simultaneously with each set of field samples tested. Most previous protocols have used 4-day tests with cadmium. Use of cadmium as a reference toxicant is also acceptable; however, ammonia is preferable because 1) it can be measured easily in the laboratory, 2) it is a confounding factor

often associated with contaminated sediments, and 3) it does not present the safety concerns and disposal issues associated with cadmium. Whichever reference toxicant is chosen, each laboratory must establish a control chart consisting of at least three tests and no more than the 20 most recent tests.

The half maximal Effective Concentration (EC50) is the concentration of a toxicant that induces a response (*i.e.*, percent mortality) that is halfway between the baseline and maximum possible effect. The EC50 for un-ionized ammonia or cadmium for each test performed should fall within two standard deviations of the mean of the previous tests on the control chart. A test falling outside two standard deviations should trigger a review of all data and test procedures to assure that the data are of good quality.

All test batches must include a negative control. The negative control should consist of sediment from the amphipod collection site or sediment as free of known contamination as possible and having previously been demonstrated to meet test control acceptability requirements. If using *R. abronius* with stations that have a grain size content of greater than 90% fines (silt + clay), a grain size control with a range of particle sizes similar to the test sediments should be included.

Each of the amphipod species has a specific tolerance to ammonia, as measured in the test chamber overlying water (Table 4.3). If any of the chambers within a test exceed this ammonia concentration, 50% of the overlying water in all chambers within the experiment may be changed up to twice per day until all are below the target concentration. The mean control survival for each test batch must be 90% or greater and each control replicate, individually, must have at least 80% survival. In addition, water quality parameters must be within acceptable limits and initial size ranges for the amphipods must be followed.

Data Analysis and Interpretation

The final response category for the test result is based on two parameters: whether or not the response is significantly different from the negative control and the magnitude of the response. Statistical comparisons between negative controls and test samples are conducted using a Student's t-test assuming unequal variance (Zar 1999).

For purposes of interpretation and comparison to response ranges, the data from test samples must be control normalized as follows:

**(mean survival of test sample / mean survival of control) * 100**

Note that the response ranges for the Nontoxic category are based on nonnormalized percent survival, but that normalized values are used for comparison to the Low, Moderate, and High response ranges. Values should be rounded to the nearest whole percentage.

After statistical analysis and normalization, the data are compared to response ranges to determine the response category for each sample. The ranges are specific to each test species and are provided in Table 4.2. The test result interpretation process is also illustrated in the form of a flow chart for each amphipod species (Figures 4.1 through 4.3).

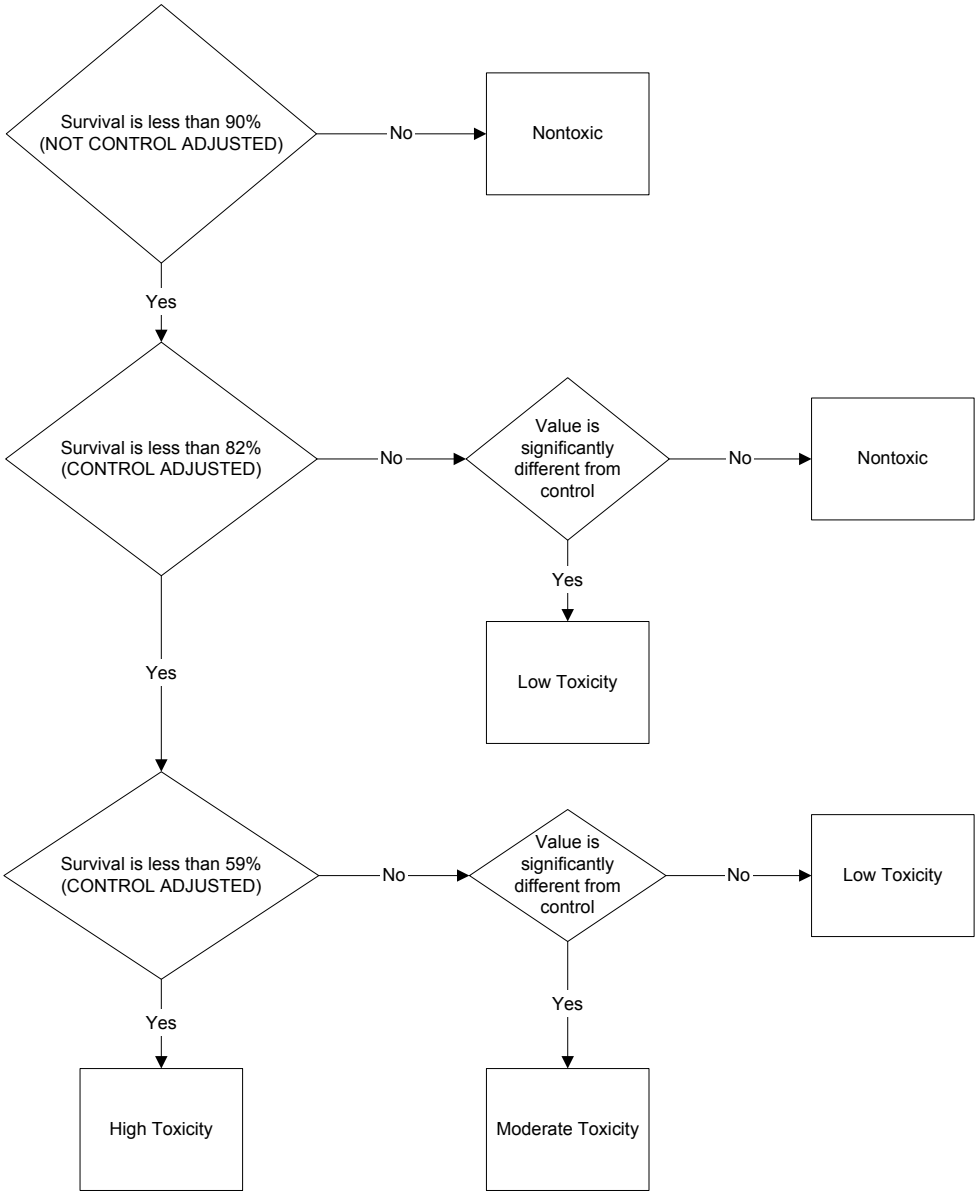


**Figure 4.1. Flow chart for determining the *E. estuarius* toxicity response category.**

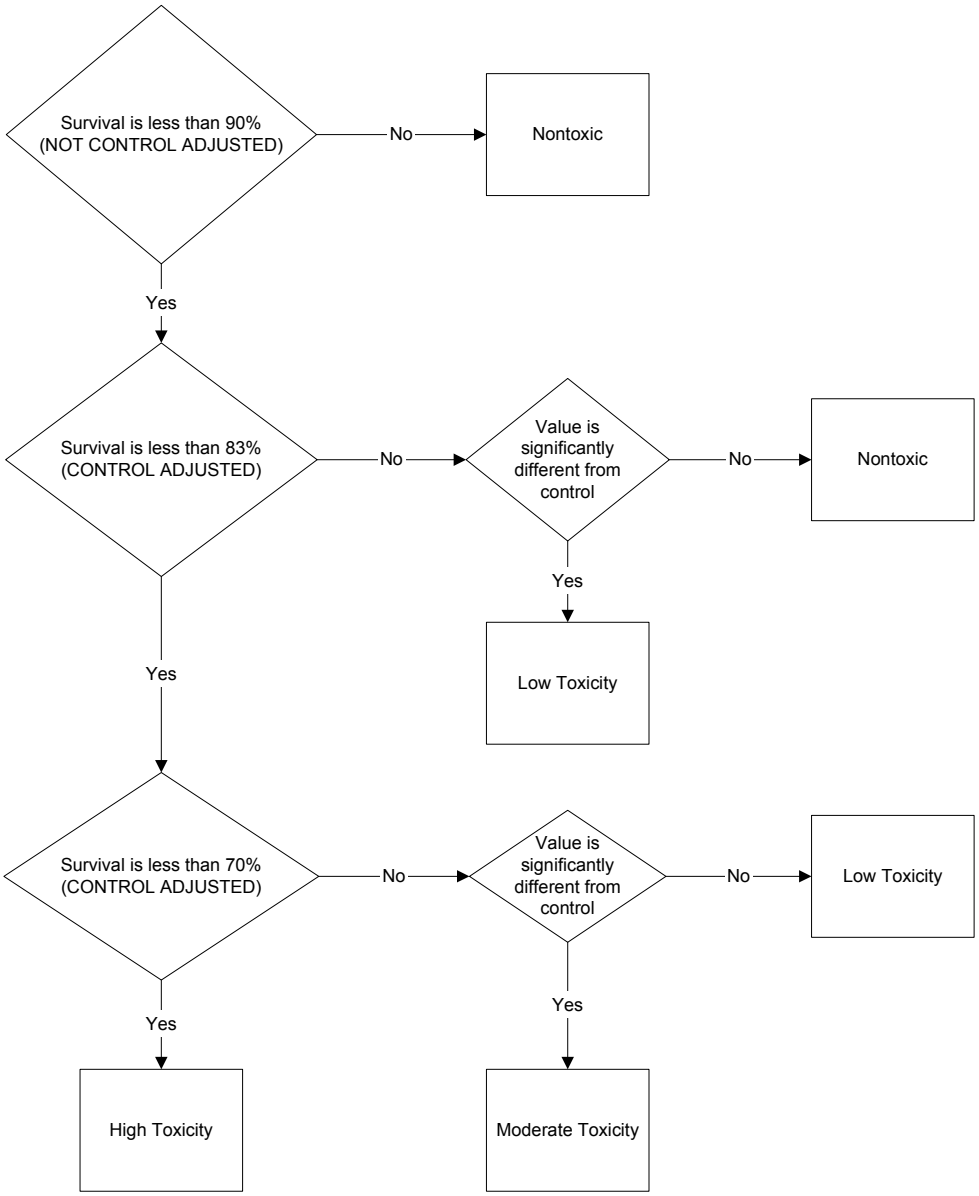


**Figure 4.2. Flow chart for determining the *R. abronius* toxicity response category.**

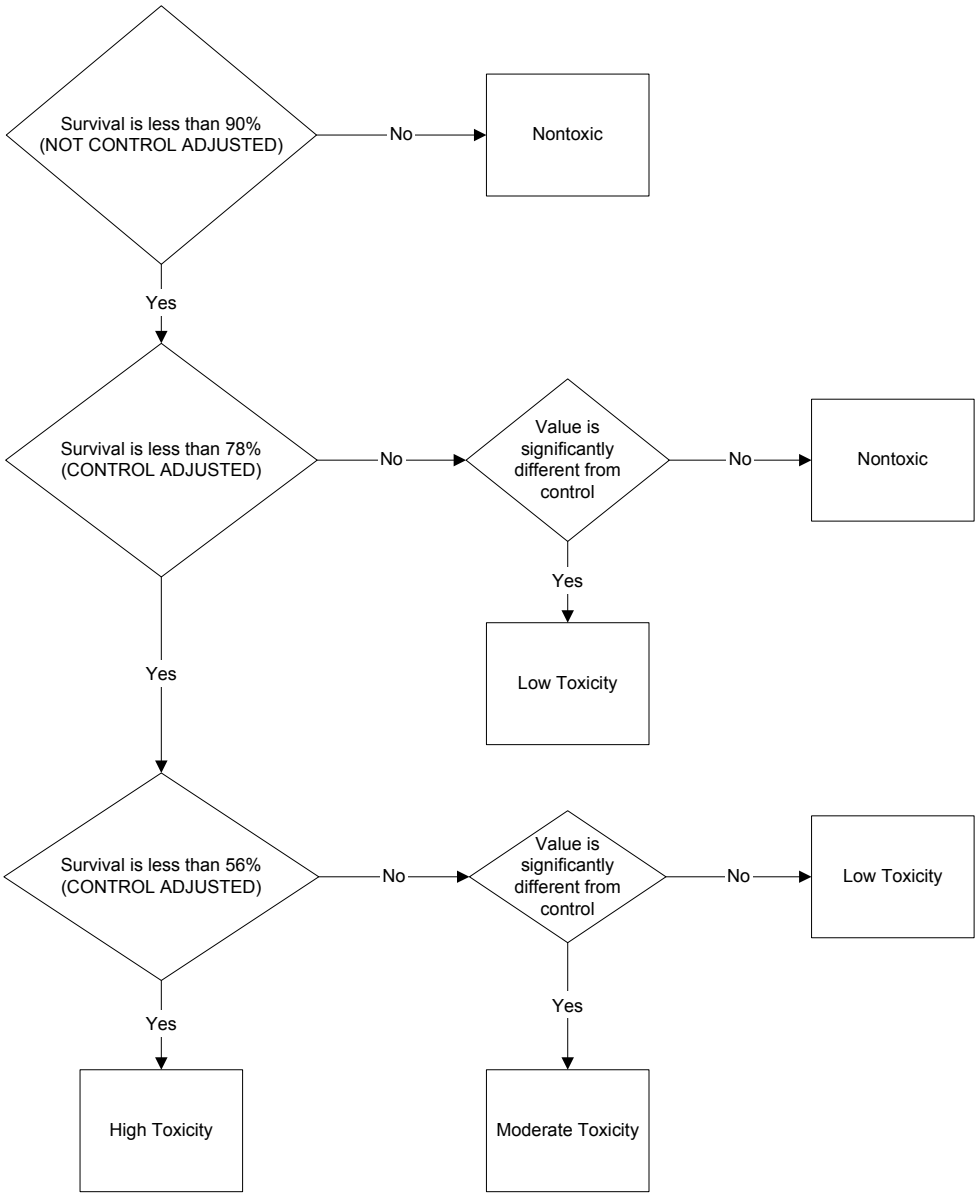


**Figure 4.3. Flow chart for determining *L. plumulosus* toxicity response category**

## Sublethal Test Methods

### *Neanthes arenaceodentata 28-day Growth and Survival*

*N. arenaceodentata* is widely distributed throughout the world in sandy or muddy sand sediments (Reish 1985). The animals live in non-permanent mucoid tubes and are deposit feeders on sediment particles (Bridges and Farrar 1997). *Neanthes* can be cultured in the laboratory and are raised commercially for use in toxicity tests. Instructions for maintaining a laboratory culture can be found in ASTM (2002a).

*N. arenaceodentata* has been used for sediment toxicity testing for about 40 years (Reish 1985). Testing methods include both 10-day survival tests and longer term exposures with growth and/or reproduction components. A 20-day version of the growth test has been used in the state of Washington for many years (PSWQA 1995). A 28-day exposure method has been developed with modifications to make the test more sensitive and reliable (Bridges and Farrar 1997, Bridges *et al.* 1997, Gardiner and Niewolny 1998, Lotufo *et al.* 2000). The recommended 28-day method is described below**.**

The 28-day *Neanthes* method recommended for the CASQO program is a revision of guidance published by ASTM (2002b). Details of the method are described in Steevens *et al.* (2008). The following method description is based on these two publications. The major modifications from the ASTM version are:

- Utilizion of <seven-day-old, post-emergent juveniles instead of two- to three-week-old worms. Initiating with younger juveniles was found to increase the sensitivity of the test (Bridges and Farrar 1997).
- Reduction of the exposure chamber volume from1 L to 300 ml. This change increased the manageability of the test by decreasing both sediment and overlying water volume requirements.
- Reduction of the number of worms per chamber from five to one. Fewer animals per replicate decreased intra-chamber variability in organism size and reduced the overall number of worms needed to conduct a test.
- Increase in the number of replicates per treatment from 5 to 10. Greater replication increased the statistical power of the test.

Test Method

The day prior to starting an experiment, approximately 75 ml of homogenized sediment should be added to 12 replicate 300 ml tall-form beakers to obtain the required depth of 2 cm. Note that two of the beakers will be used for sediment pore water ammonia measurements and therefore will not have animals added to them. The sediment is then overlain with 125 ml of 30 g/kg seawater. Either natural 0.45-µm filtered seawater or artificial seawater may be used. Beakers are then gently aerated and maintained at 20°C and with a light cycle of 12:12 hours light:dark. A listing of all parameters for the *Neanthes* test can be found in Table 4.4.

**Table 4.4. Test characteristics for 28-day *Neanthes arenaceodentata* growth and survival test.**

| Parameter | *N. arenaceodentata* |
|---|---|
| 1. Temperature | 20 ±1°C |
| 2. Salinity | 30 ±2 g/kg |
| 3. Illuminance | 500 - 1000 lux |
| 4. Photoperiod | 12:12 hours light:dark |
| 5. Acclimation | 1 day at test temperature and salinity |
| 6. Size and life stage | ≤7 days post-emergent juveniles |
| 7. Number of organisms/chamber | 1 |
| 8. Number of replicates/treatment | 10 |
| 9. Aeration | Enough to maintain 90% saturation |
| 10. Water quality measurements | Temperature daily. pH, salinity, ammonia and DO of overlying water at $T_0$ and $T_{final}$. Prior to each water change pH, salinity, ammonia and DO of overlying water in 3 replicates per treatment. Pore water pH, salinity, ammonia at $T_0$ and $T_{final}$ from surrogate beakers. |
| 11. Feeding | Twice per week. 2 mg of Tetramarin® on one day and 2 mg of Tetramarin® plus 2 mg of alfalfa on the other. |
| 12. Test acceptability criteria | Mean control survival of 80% and positive growth in controls. |
| 13. Grain size tolerance | 5 - 100% sand |
| 14. Ammonia tolerance | <20 (total, mg/L) |
| 15. Total sulfide tolerance | <5 (mg/L) |

On day 0, *N. arenaceodentata* (≤seven days post-emergence) are placed into counting chambers (one animal per chamber): one chamber for each exposure beaker plus an additional five for initial weight measurement. Counting chambers are randomly assigned to each exposure beaker and the initial weight group. The contents of each counting chamber are then gently transferred to its corresponding beaker. Animals caught in the surface tension in the exposure beakers should be sunk by gently dropping water on them.

The five animals for initial weight measurement should be rinsed in de-ionized water, placed on tared pans and dried in an oven at 60°C for 24 hours. After 24 hours in the drying oven, the pans are removed, allowed to cool in a desiccator, and then weighed to obtain initial weight for growth calculations.

Starting on day 0 of the test run, water-quality measurements (including dissolved oxygen, pH, salinity, and ammonia) are taken from the overlying water in each test chamber. These measurements should be taken in three replicates per sediment. The same measurements are repeated once weekly thereafter, always prior to water changes (see below). Exposure temperature (min/max) is also monitored and recorded daily. Observations of each replicate beaker are conducted daily and should include whether worms are on the surface of the sediment and how, if at all, sediment appearance has changed. In addition, pore water ammonia must be determined at day 0 and at the end of the exposure in the two surrogate beakers with no worms.

Water must be exchanged (~60 ml) from each beaker once per week after water quality parameters are measured. The worms are fed twice per week, separated by about three days (*e.g.*, Tuesdays and Fridays). Each beaker is provided with 2 mg of Tetramarin® (Tetra Sales, Blacksburg, Virginia) one day and 2 mg of Tetramarin® plus 2 mg of alfalfa on the other. Both the Tetramarin® and the alfalfa are ground to 0.5 mm and are delivered to the exposure containers in a seawater slurry.

On day 28, the sediment contained in each beaker is gently sieved (using a 425-µm-mesh sieve) and surviving worms are recovered. Surviving worms are counted and recorded. Survival is determined by gently prodding animals with a blunt probe. If movement is observed, the animal is considered to be alive. Worms that are unaccounted for are considered to be dead. Surviving animals in each replicate should then be rinsed with de-ionized water, put on pre-weighed pans and placed in a drying oven at 60°C for 24 hours. After 24 hours in the drying oven, the pans are removed, allowed to cool in a desicator then weighed to obtain the individual dry weight for each replicate/animal to the nearest 0.1 mg.

Quality Assurance

A 4-day, water-only reference toxicant test using ammonia should be performed simultaneously with each set of field samples tested. Each laboratory must establish a control chart consisting of at least three tests and tracking no more than the 20 most recent. The EC50 for each test performed should fall within two standard deviations of the mean of the previous tests on the control chart. A test falling outside two standard deviations should trigger a review of all data and test procedures to assure that the data are of good quality.

All test batches must include a negative control. The negative control should consist of sediment as free of known contamination as possible and having previously been shown to meet test control acceptability requirements.

Total ammonia concentrations above 20 mg/L have been found to have a negative impact on both survival and growth (Dillon *et al.* 1993). If pore water ammonia concentration, as measured in the surrogate beakers, in any sediment treatment (station) is greater than 20 mg/L, then all chambers should undergo up to twice daily 50% water changes until all treatments fall below this level. Addition of animals cannot occur until acceptable ammonia concentrations are present.

The mean control survival for each test batch must be 80% or greater, and there must be measurable positive growth in the controls. In addition, water quality parameters should be within acceptable limits.

Data Analysis and Interpretation

Growth rate is calculated using the equation:

$$G = \frac{DWTt_2 - DWTt_1}{T}$$

Where: $DWTt_2$ = the mean dry weight (mg) of surviving animals in a treatment at test termination;
$DWTt_1$ = the mean dry weight of the initial group of animals; and
T = the duration of the test in days. The growth rate is therefore expressed in units of mg/day.

Statistical comparisons for the growth endpoint are achieved using a Student's t-test assuming unequal variance (Zar 1999). For purposes of interpretation and comparison to response ranges, the data from test samples must be control normalized for growth as follows:

**(mean growth of test sample / mean growth of control) * 100**

After statistical analysis and normalization, the data are compared to the ranges in Table 4.2 to determine the response category for each sample. Note that the *Neanthes* test is the only method in this document where control normalized data is used to determine whether the response is classified as Nontoxic. Figure 4.4 illustrates the data interpretation process in the form of a flow chart.

Survival is calculated in each treatment group by dividing the number of surviving animals by the number of animals at the start. Statistical comparisons between negative controls and test samples for the survival endpoint must use categorical statistics since there are only two possible outcomes per replicate, dead or alive. Fisher's exact test is the method used for the survival endpoint (Zar 1999). The survival endpoint is not used for the toxicity response classification, but can be used as ancillary data in assessment of data quality and sediment condition.

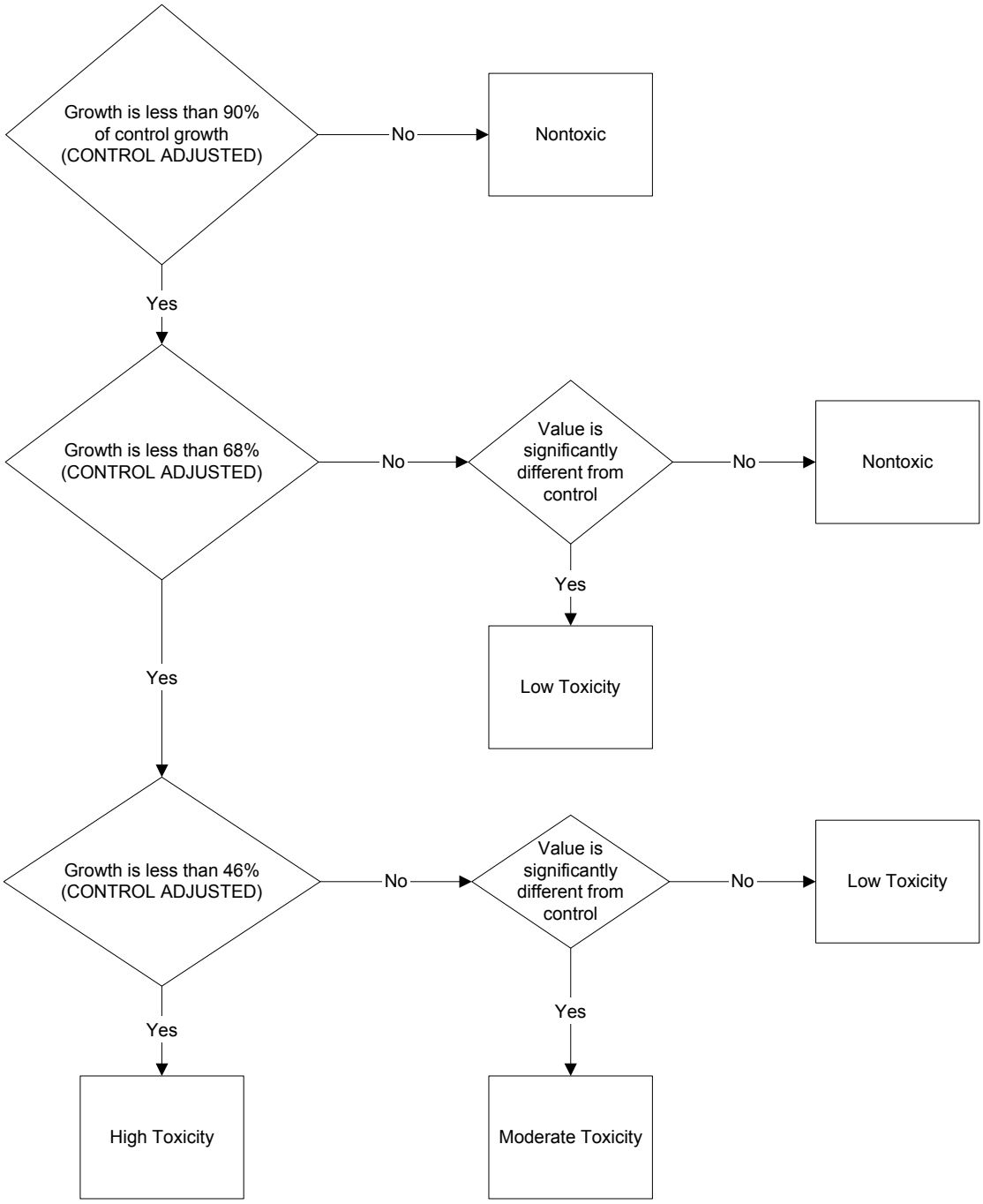


**Figure 4.4. Flow chart for determining *N. arenaceodentata* toxicity response category.**

***Mytilus galloprovincialis 2-day Embryo Test at the Sediment-Water Interface***

Use of embryos from the mussel *M. galloprovincialis* for toxicity testing is common in California and is part of the USEPA West Coast methods (USEPA 1995). However, most of these tests have been performed on aqueous samples, as opposed to sediment. The method described here is a modification of the exposure apparatus to allow for testing sediment from intact core samples at the interface between the sediment and overlying water, or SWI. This method has been used in a regional monitoring program in San Francisco Bay (SFEI 2001).

Contaminants in the sediment can be an important source of toxicity to the water column (Burgess *et al.* 1993). This flux of contaminants out of the sediment would be expected to have its greatest effect on toxicity where the sediment and overlying water meet. Therefore, the method described here measures an important component of sediment toxicity that is usually not investigated.

Details of the exposure system can be found in Anderson *et al*. (1996) and methods for the preparation and handling of the mussel embryos are in USEPA (1995). A listing of all test parameters can be found in Table 4.4.

**Table 4.4. Characteristics for 2-day mussel (*Mytilus galloprovincialis*) embryo development test at the sediment-water interface.**

| Parameter | *Mytilus galloprovincialis* |
|---|---|
| 1. Temperature | 15 ±1°C |
| 2. Salinity | 32 ±2 g/kg |
| 3. Illuminance | 500 - 1000 lux |
| 4. Photoperiod | 16:8 hours light:dark |
| 5. Acclimation | 2 days at test temperature and salinity; up to 4 weeks |
| 6. Size and life stage | Newly fertilized eggs |
| 7. Number of organisms/chamber | ~ 250 |
| 8. Number of replicates/treatment | 4 |
| 9. Aeration | Enough to maintain 90% saturation |
| 10. Water quality measurements | Temperature daily; pH, salinity, ammonia and DO of overlying water at $T_0$ and $T_{final}$ from surrogate core tube |
| 11. Feeding | None |
| 12. Test acceptability criteria | Mean control percent normal-alive of ≥80%; mee*t al*l water quality limits |
| 13. Grain size tolerance | 0 - 100% sand |
| 14. Ammonia tolerance | <4 (total, mg/L) |
| 15. Total sulfide tolerance | <0.09 (mg/L) |

Test Method

Sediment is generally collected in polycarbonate core tubes (7.5 cm diameter) with polyethylene caps. A 5-cm depth of sediment is collected. There must be at least 8 cm between the top of the sediment and the top of the core tube in order to allow room for the screen tube that will hold the embryos for the test. A minimum of four cores should be collected for toxicity testing from each station. At least one additional core should be collected for water quality measurements. Intact cores should be transported with overlying water from the sediment collection in place. Approximately 24 hour prior to test initiation, all but about 0.5 cm of the overlying water should be siphoned off and gently replaced with 300 ml of clean seawater. The core tubes are then placed at 15°C with gentle aeration.

Field collection of sediment cores (e.g., from a grab sample) is preferred, because this provides the most undisturbed sample for testing. However, homogenized sediment samples may also be used. If the latter approach is taken, the homogenized sediment should be loaded into the test chambers in the laboratory, as described below, in order to simulate the core sample. The maximum holding time for homogenized sediment used in the SWI test is 4 weeks. However, it is highly recommended that SWI tests be initiated within 14 days of sediment sampling.

If homogenized sediments are to be tested, the sediments should be thoroughly press sieved with a 2-mm stainless steel sifting screen following homogenization. After sieving, 5 cm of sediment is added to the same type of core tube used for field collection. Then 300 ml of 32 ppt, 15°C seawater should be added. Approximately 2 cm of free space should be left at the surface to accommodate displacement due to eventual inclusion of the aerator and screen tube in the test chamber. Sediment should be allowed to settle and equilibrate 24 hours before initiation of test.

On the day of test initiation, polycarbonate screen tubes with 37-μm mesh are gently added to each core tube (Figure 4.5). Details of screen tube construction can be found in Anderson *et al*. (1996). When the tubes are placed on the sediment, the bottom collar should rest so that the screen is suspended approximately 1 cm above the substrate. The water level outside the tube should be approximately 0.5 cm below the top of the screen tube. If necessary, water may need to be siphoned from the outside of the screen tube to achieve the proper level. Once the test chamber has been set up, there should be about 150 ml of water inside the screen tube. At this point, the aeration should be directed inside the screen tube.

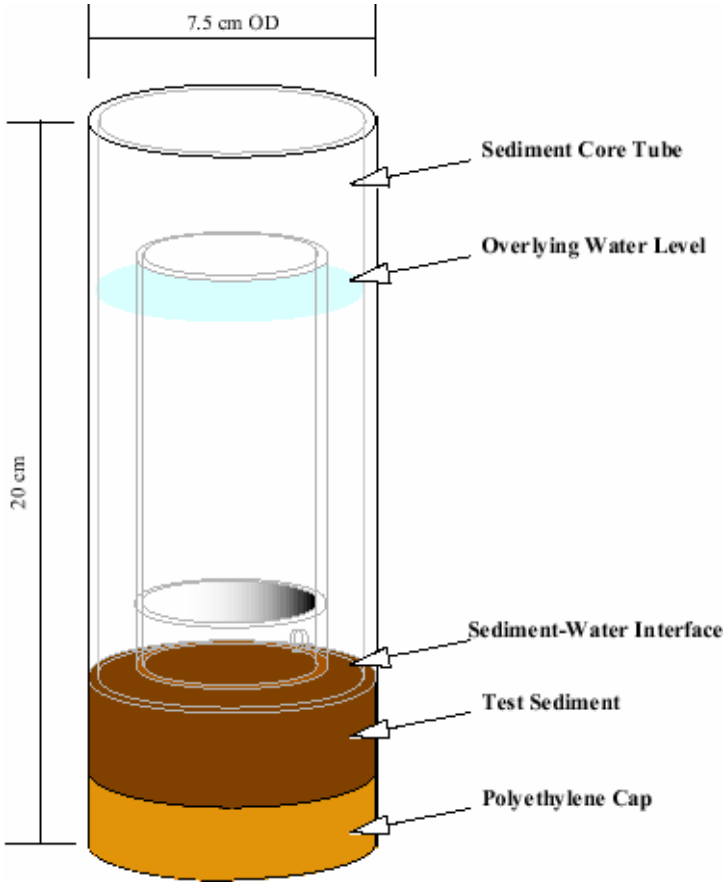


**Figure 4.5. Schematic diagram of sediment-water interface exposure system (Anderson *et al*. 1996).**

Mussel brood stock can be obtained from commercial vendors or collected from the field in areas known to be free of contaminants. Adult mussels should be acclimated to laboratory conditions for at least two days prior to testing and may be held in the laboratory for up to four weeks. Fertilized mussel eggs are prepared as described in the EPA manual (USEPA 1995). Approximately 250 embryos are introduced to the screen tube of each replicate. It is important to add the same number of embryos to each replicate. An additional 5 scintillation or shell vials with 10 ml of seawater must be prepared, and the same volume of embryo stock added to these containers. These additional samples are used to determine the initial quantity of embryos. The initial samples should be preserved immediately and counted using a microscope.

Water quality parameters (dissolved oxygen, pH, salinity and ammonia) should be made prior to test initiation on day 0 and at test termination. Temperature should be monitored continuously. Daily observations should be made on each replicate with special attention to aeration, sediment

condition (*e.g.*, anoxia, microbial growth such as a bacterial/diatom mat) and the presence of any invertebrates in the sediment cores.

At the end of the exposure period, the screen tubes are removed from the sediment and the embryos are washed into glass scintillation or shell vials with seawater squirt bottles. Care must be taken to recover all embryos from the screen. Preservative is then added to the vials and the embryos are examined microscopically to determine if they are normally developed. An inverted microscope is recommended. This allows for viewing the embryos through the bottom of the vial and is thus faster than using a Rafter cell. This approach has the additional advantage of not exposing technicians to preservative fumes. When evaluating the embryos with the microscope, all embryos present in each vial must be observed and scored as normally or abnormally developed. Normally developed embryos have a distinctive "D" shape (Figure 4.6). Embryos not possessing this shape are scored as abnormal. Embryos that appear normal but do not contain internal tissues are also counted as normal but it is recommended that they be enumerated separately.

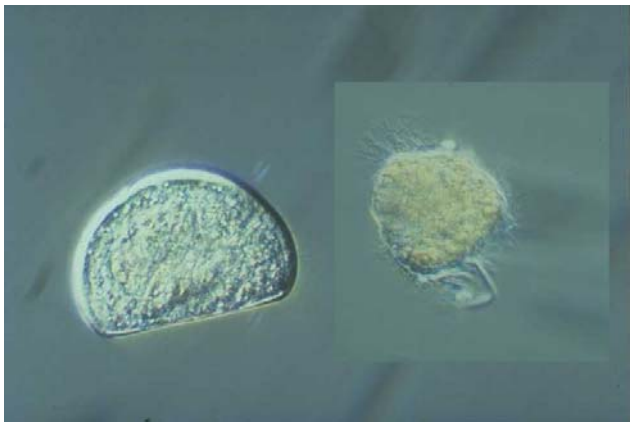

**Figure 4.6. Normal (left) and abnormal (right) *M. galloprovincialis* embryos after 48 hours of development.**

Quality Assurance

A 2-day, water-only reference toxicant test using ammonia or copper should be performed simultaneously with each set of field samples tested. Each laboratory must establish a control chart consisting of at least three tests and no more than the 20 most recent. The EC50 for each test performed should fall within two standard deviations of the mean of the previous tests on the control chart. A test falling outside two standard deviations should trigger a review of all data and test procedures to assure that the data are of good quality.

*M. galloprovincialis* embryos are quite sensitive to ammonia. The water overlying the sediment in the core tube cannot have a total ammonia concentration exceeding 4 mg/L at the start of an exposure. If any station within an experiment exceeds this level, then the overlying water in all exposure chambers in the test batch should be replaced until none exceed this level.

All test batches must include both chamber and seawater negative controls. The chamber negative control should consist of seawater in a core tube with no sediment and a screen tube placed inside. This control tests for any toxicity associated with the exposure system. The seawater negative control usually consists of seawater inside a scintillation or shell vial. This control verifies the health of the organisms. The control from the simultaneous reference toxicant exposure may serve for the seawater negative control.

Care must be taken that the correct species is being used. It has been found that there are differences in sensitivity between *M. galloprovincialis* and the other commonly used species *Mytilus edulis* (Bryn Phillips, personal communication). Organisms should be identified to species by competent personnel using morphological characteristics and appropriate keys. Animals purchased from culture facilities are assumed to be the correct species.

The mean control percent normal-alive for each test batch must be 70% or greater. In addition, water quality parameters must be within acceptable limits.

Data Analysis and Interpretation

The reported endpoint for this method in this document is different from that which is stated in the USEPA manual (USEPA 1995). The endpoint determined for this method is calculated as follows:

**(# of normal embryos/initial # added) * 100**

The results are expressed as percent normal-alive (PNA). This endpoint takes into account the difficulty in finding abnormal embryos microscopically among the sediment grains that are usually carried into the vial when the embryos are rinsed from the screen tube. The assumption is also made that missing and abnormal embryos are not alive at the end of the exposure. Note that by counting the abnormal embryos as well, the traditional percent normal can also be calculated.

Statistical comparisons between negative controls and test samples are achieved using a Student's t-test assuming unequal variance (Zar 1999). For purposes of interpretation and comparison to established response ranges, the data from test samples must be control normalized:

**(mean PNA of test sample/mean PNA of control) * 100**

After statistical analysis and normalization, the data are compared to response ranges to determine the toxicity response category for each sample (Table 4.2; Figure 4.7). Note that non-normalized values are used for determining the Nontoxic category, and normalized values are used for comparisons to the other category ranges.

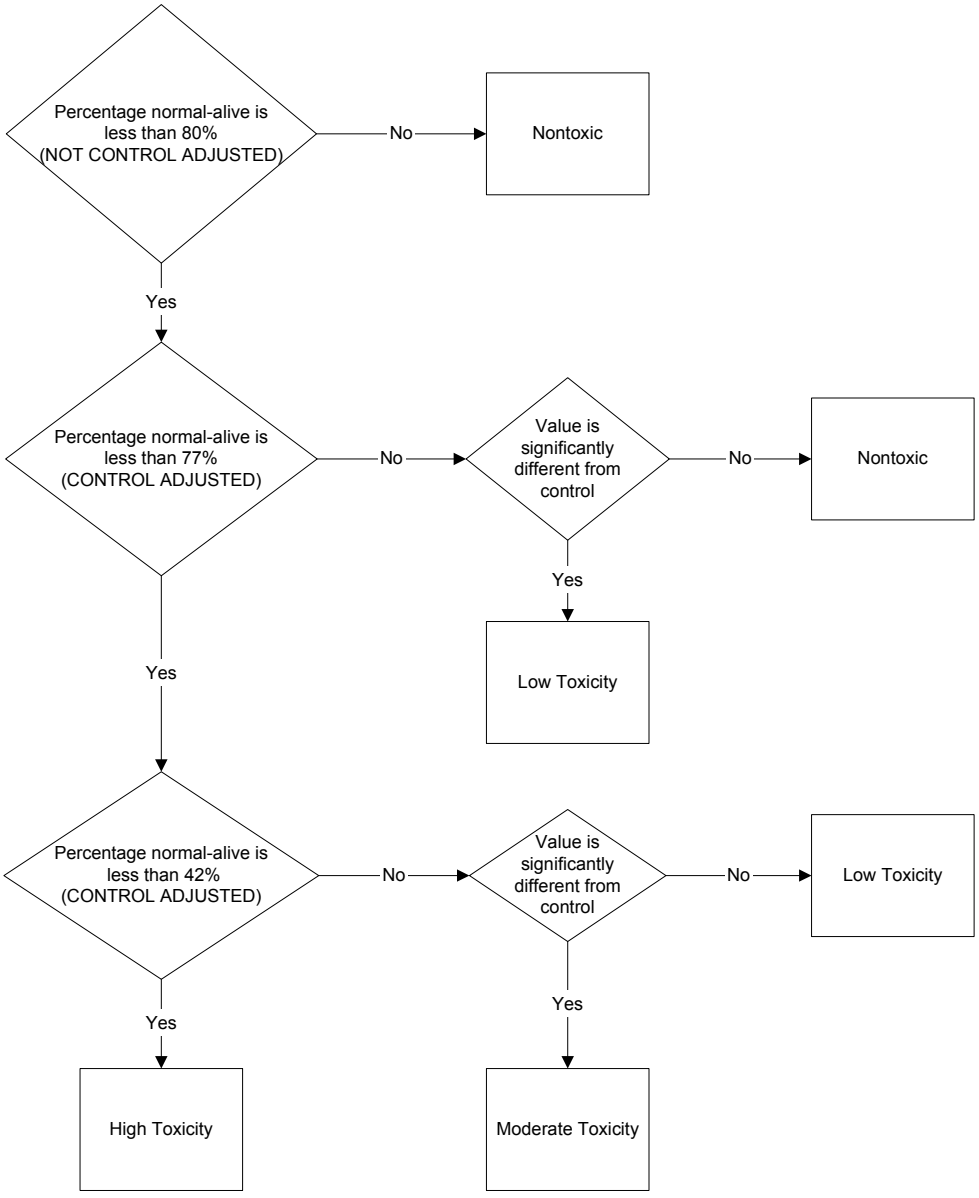


**Figure 4.7. Flow chart for determining *Mytilus* embryo development at the SWI response category.**

## Integration of Toxicity Test Results

To determine the Toxicity LOE category for a given station, the results of the individual toxicity tests must first be transformed into numeric values. Numeric category scores are assigned to each toxicity test result as follows: Nontoxic = 1, Low Toxicity = 2, Moderate Toxicity = 3, High Toxicity = 4. The scores of all tests are then averaged to yield the station's Toxicity LOE category, with each test result weighted equally in the calculation.

If calculated means have decimal values of 0.5 or higher, they are rounded up to the nearest category. If means have decimal values of 0.5 or less, they are rounded down. The scheme relating numeric scores with category names is the same for the Toxicity LOE as for the individual tests (e.g., Moderate Toxicity = 3).

## Data Management

Data should be collected and formatted in such a manner that it can be incorporated into a regional sediment quality database. Examples of database formats can be obtained from the Surface Water Ambient Monitoring Program (SWAMP), CASQO, and Regional Monitoring Program (RMP). The SWAMP data format for toxicity is currently being drafted; information on this database can be found at http://mpsl.mlml.calstate.edu under the, "How to be Comparable with the SWAMP Database" web page. The CASQO database can be found on the Data Catalog page of the SCCWRP website (http://www.sccwrp.org/view.php?id=422). This also includes a user's guide that provides help with data formatting. Information on the RMP database format is available through www.sfei.org.

The electronic data records must include the following:

- Station and sample collection information
- Toxicity raw and summarized data
- Statistical results
- Water quality data collected during toxicity testing

In addition it is important to keep records regarding any anomalies that occur during testing (*e.g.*, power failure or why a replicate was missing). These records may help with data interpretation and should be included in comments fields within the database.

## Example of Toxicity Line of Evidence Calculation

### *Data Preparation*

The raw data from at least two toxicity test methods are compiled and the mean response (*e.g.*, % survival) for each sample is calculated. The response data must be control normalized ((data from assessment station/control data) * 100). T-tests must be performed on the raw data from the assessment station versus control response. A sample data set containing results from two tests,

the amphipod *E. estuarius* survival test and sediment-water interface test using the mussel *M. galloprovincialis* embryo development, is shown in Table 4.5.

**Table 4.5. Toxicity data used in the example.**

| Test Method | *E. estuarius* survival | *M. galloprovincialis* Percent Normal Alive |
|---|---|---|
| Raw Station Response | 90% | 57% |
| Raw Control Response | 92% | 92% |
| Control Normalized Response | 98% | 62% |
| Statistical Difference from Control | No | Yes |

### *Individual Toxicity Test Result Classification*

The data from each toxicity test are compared to a series of response ranges that are unique to each test method (Table 4.2). Note that in the case of *Eohaustorius* and *Mytilus*, for the Nontoxic category, the *non-normalized* mean response for the assessment station is compared to the range, whereas for the Moderate and High toxicity categories, the *control-normalized* response is compared to the ranges. Figures 4.8 and 4.9 show the data classification results for each test organism. The toxicity category is based on both the response level and whether a statistically significant difference is present. The raw *Eohaustorius* survival value of 90% classifies that test in the Nontoxic category (Figure 4.8) and the *Mytilus* percent normal-alive value of 62% (control normalized) classifies that test in the Moderate Toxicity category (Figure 4.9).

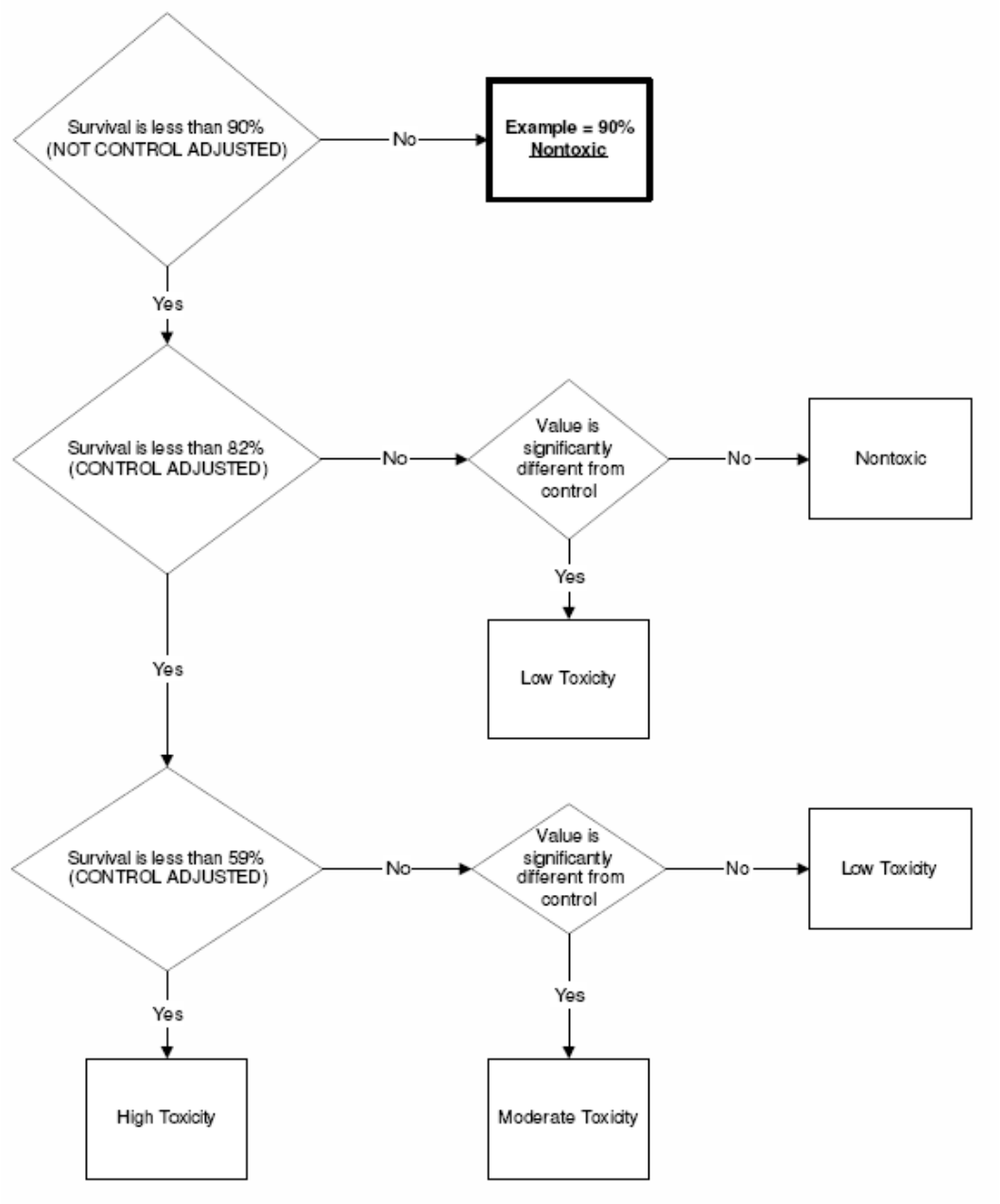


**Figure 4.8. Flow chart for assignment of toxicity response category for *E. estuarius* example data.**

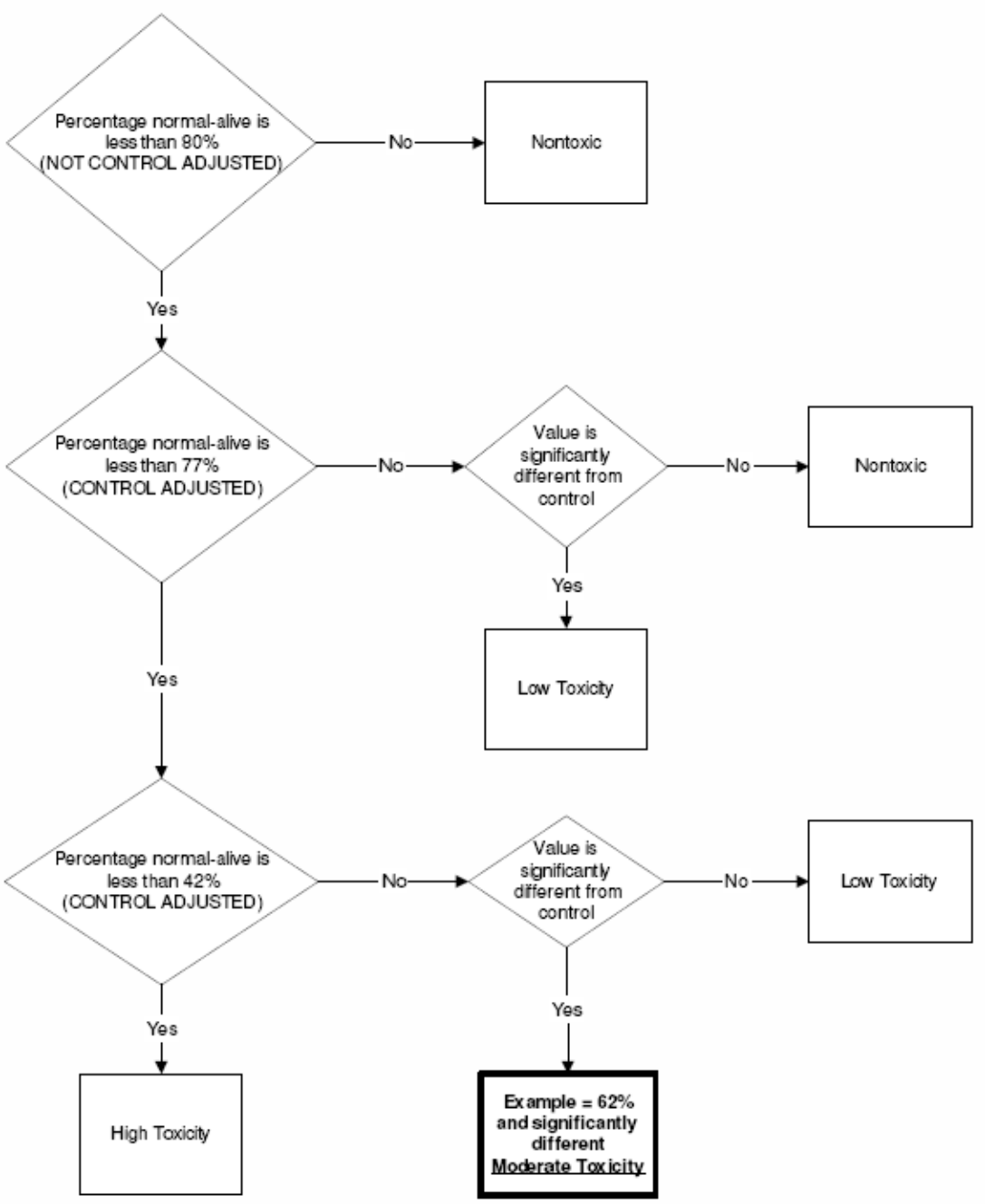


**Figure 4.9. Flow chart for assignment of toxicity response category for *M. galloprovincialis* example data.**

***Integration of Toxicity Test Results***

The final step in determining the Toxicity LOE is to integrate the toxicity test results. This is accomplished by assigning numeric category scores for each test result (Nontoxic = 1, Low Toxicity = 2, Moderate Toxicity = 3, High Toxicity = 4). The arithmetic mean of all tests corresponds to the Toxicity LOE category. Means with decimal values of 0.5 and higher are rounded up to the nearest category. Means with decimal values of less than 0.5 are rounded down.

For the example data, the *Eohaustorius* result is classified as Nontoxic (score = 1) and the *Mytilus* result is classified as Moderate Toxicity (score = 3). The mean category score for the two toxicity tests for this station is 2, which corresponds to the Low Toxicity category for the Toxicity LOE.

# CHAPTER 5: BENTHIC COMMUNITY COMPOSITION

The composition of the benthic community constitutes an essential LOE for sediment quality assessment. The Benthic LOE is a direct measure of the effect that sediment contaminant exposure has on the benthic biota of California's bays and estuaries. Determination of the Benthic LOE is based on four measures of benthic community condition: 1) the Index of Biotic Integrity (IBI), 2) the Relative Benthic Index (RBI), 3) the Benthic Response Index (BRI), and 4) the River Invertebrate Prediction and Classification System (RIVPACS). This chapter includes computational tools for calculating the Benthic LOE category and provides an example of the step-by-step process for its determination.

## Objectives

The goal of this chapter is to provide recommendations for laboratory processing, quality assurance (QA), quality control (QC), and data analysis procedures that are recommended for assessing the condition of soft bottom benthic macroinvertebrate communities of California's bays and estuaries. It is intended to supplement protocols presently used in California with regard to methods that meet the requirements of the sediment quality assessment framework contained in the draft sediment quality objectives (SQO) policy.

## Scope

This chapter describes laboratory procedures recommended for the processing of benthic infauna samples and data analysis methods for use in the California sediment quality objectives (CASQO) program. All key aspects of sample processing are described, including sample preservation, sorting, taxonomic analysis, quality assurance/quality control (QA/QC), and data analysis. A high level of detail regarding methods and benthic indices are included in this document because many of these methods are new and/or few guidance documents are available.

Efficient sample sorting and accuracy in taxonomic identification are critical to obtaining high quality results. Species identification requires a high level of expertise by qualified taxonomists and there is always the potential for inaccurate results due to changes in nomenclature or subjective interpretation of diagnostic characteristics. Consequently, this chapter contains detailed recommendations for assuring the quality of sample processing. Example forms for recording the results of QA/QC activities are also provided in the appendices.

## Sample Processing

Benthic sample processing in the laboratory includes the following tasks (Figure 5.1):

- Sample Preservation: The sample is washed free of formalin fixative and transferred into an alcohol solution for processing and storage.

- Sorting: Organisms are removed from sample debris, sorted into taxonomic groupings to facilitate subsequent taxonomic analysis, and sorting quality is evaluated and corrected if deficient.
- Taxonomic Analysis: Organisms in samples are identified and counted, voucher specimens are prepared to document identifications, and taxonomic analysis accuracy may be evaluated by reanalyzing selected samples.
- Data Entry: Taxonomic analysis and quality control results are recorded.
- Data analysis: The habitat type is determined and the taxonomic analysis data are processed to determine the Benthic LOE category for each sampling site.

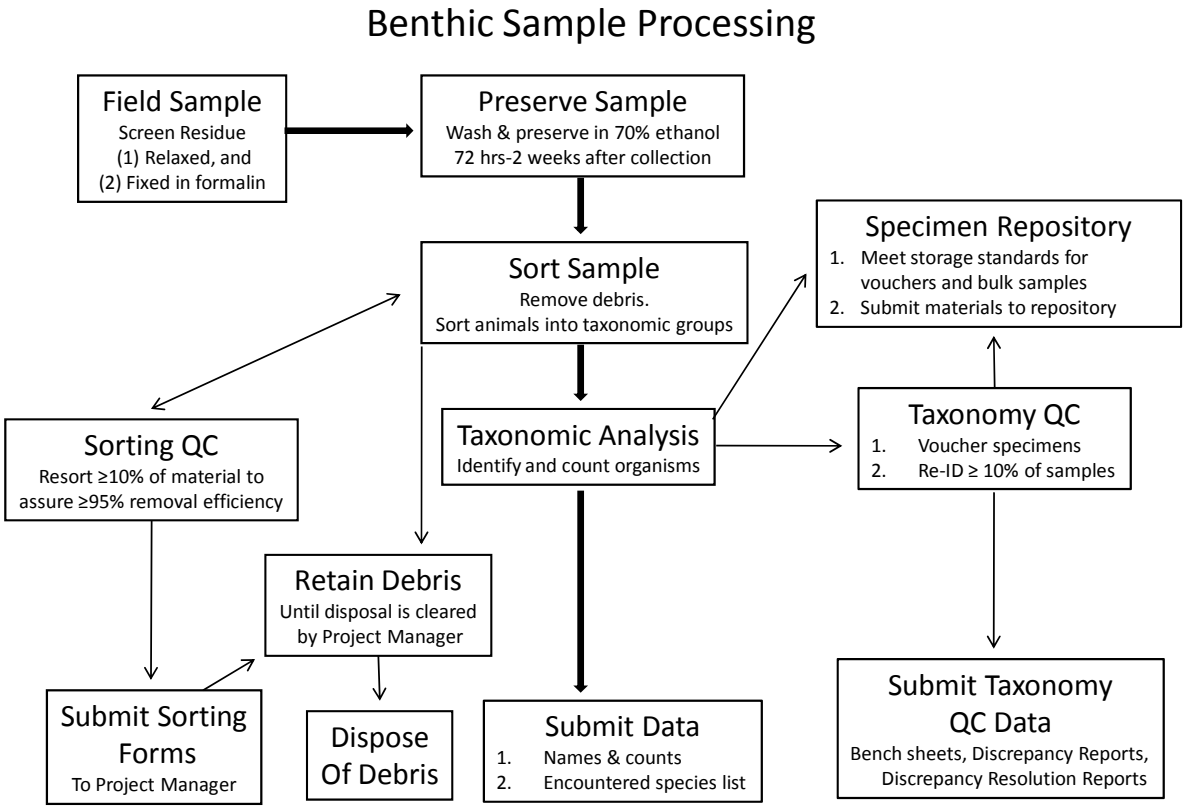


**Figure 5.1. Overview of laboratory processing for benthic community samples.**

## Sample Preservation

Samples that are received from the field in formalin fixative must be washed and transferred to alcohol 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 molluscs) which are often essential for accurate identifications. Secondly, formaldehyde is a noxious, potentially dangerous chemical. Replacing formaldehyde with ethanol makes subsequent sample handling safer. Other benefits of the washing process are the removal of excess silt from mud balls and fecal pellets that may have broken down during fixation and, in some cases, the opportunity to separate most of the organisms in a sample from inorganic debris using an elutriation process (defined below).

Samples fixed in formalin in the field should remain in formalin fixative for at least 72 hours, but no sample should remain in fixative for longer than two weeks because formalin will decalcify of molluscs and echinoderms. Benthic community samples should be preserved in a 70% ethanol solution. Denatured alcohol and dyes for staining organisms are not recommended. The alcohol preservative should be buffered with marble chips, especially if the ethanol is produced by industrial distillation rather than fermentation. Ethanol is commonly purchased as a 95% ethanol solution. To prepare 1 L of 70% ethanol solution, 263 ml of purified water (*e.g.*, filtered and de-ionized by reverse osmosis) is added to 737 ml of 95% ethanol. If samples contain a high percent of crustaceans, it is recommended to substitute some water with glycerine (e.g., 70% ethanol, 25% purified water, 5% glycerine) to help maintain exoskeleton shape.

## Sample Sorting

Sorting is the process by which organisms in a benthic sample that were alive at time of collection are removed from the organic and inorganic residues (debris) that compose the sample, and sorted into broad taxonomic categories for subsequent analysis by taxonomists. Sorting must be accurate and complete to assure the value of subsequent steps in the sample analysis process. Quality control procedures (see below) are used to assure that sorting accuracy and completeness meet data quality objectives (DQOs).

Several sorting techniques are used for the removal of benthic organisms from sediment. Commonly, a small amount of sample is placed in a Petri dish and each organism is systematically sorted and removed under a dissecting microscope using forceps. The "elutriation" or "floating" method is a technique that is effective when a sample is primarily coarse sand or highly organic. Inorganic material in the sample is separated from the lighter organic debris and organisms by the following elutriation process: After washing the formalin from the sample, spread the sample material out in a shallow pan or flat tray and cover with water. Gently agitate the sample by hand to allow the lighter fraction of debris and organisms to separate from the heavier material. The densest material settles to the bottom while the less dense material, such as organic material, arthropods, and other soft-bodied organisms, becomes suspended. The solution is then poured through the sieve and sorted. The denser material (*e.g.*, sand grains and molluscs) is covered with water, so that it is more easily sorted and removed under a dissecting microscope. The water containing the lighter material should be decanted through a sieve, repeating the process several times until no more material is observed in the

decanted water. Then the material in the decanted water is collected into a small sample container, topped with preservative, and returned to the original sample container along with the balance of the sample material. The sample container should be filled with preservative and its lid tightly affixed. Both containers should be labeled properly with internal labels.

It is generally recommended that sorting be done in 70% ethanol, with care taken to assure that the sample being sorted is always fully covered with alcohol. It is not uncommon for Ophiuroidea to be removed from the ethanol and air dried to assist with identification. Organisms removed from the sample are sorted into taxonomic lots for subsequent taxonomic analysis. 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. 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 one-half hour) required to sort the sample should be recorded on the sorting record form (Appendix C).

Aggregate the taxa lots into one or more sample containers. It is generally recommended that each sample container and taxa lot be internally labeled with station name, sampling date and depth, and split number (if more than one container is used). Labels should be written in pencil or indelible ink on 100% rag-paper, poly-paper, or other paper suitable for permanent wet labels.

A breakdown of taxonomic lots is provided in Table 5.1. The purpose of the taxonomic lots is to facilitate taxonomic analysis by project taxonomists, with each lot being analyzed by a single taxonomist. Therefore, the specifics of taxonomic lots may vary with the number of project taxonomists available and the details of their taxonomic expertise. In Southern California Bight Regional Monitoring Projects, taxonomic lots are usually the same as those into which identified and enumerated materials are stored (Table 5.1).

**Table 5.1. Taxonomic lots for Southern California Bight regional monitoring projects.**

| Annelid Lots | Arthropod Lots | Mollusc Lots | Echinoderm Lots | Misc. Phyla Lots |
|---|---|---|---|---|
| Oligochaeta | Ostracoda | Bivalvia | Ophiuroidea | Cnidaria |
| Spionidae | Amphipoda | Gastropoda | Misc. Echinodermata | Nemertea |
| Cirratulidae | Isopoda | Misc. Mollusca | | Other Phyla (a collective lot) |
| Misc. Polychaetes | Decapoda | | | |
| | Misc. Arthropoda | | | |

### *Quality Control*

Quality control of sorting is essential to assure the value of all the subsequent steps in the sample analysis process. A standard sorting form (Appendix C) is usually 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 QC purposes. It is good practice to have, at a minimum, 10 to 20% of all samples re-sorted to monitor sorter performance.

There are two recommended approaches used for re-sorting: the aliquot sample method, and the whole sample method. A laboratory may choose one of these two methods but, for consistency, a single method should be employed by a laboratory for all samples in a single project. The re-sort method used should be noted on the sorting form along with the re-sort results.

Whole Sample Method:

At least 10% of the samples processed by each sorter are completely re-sorted.

Aliquot Method:

A representative aliquot of at least 10% of the sample volume of every sample processed by each sorter is re-sorted.

Regardless of the method employed, an experienced sorter other than the original sorter conducts all re-sorting and percent sorting efficiency is calculated as follows:

Whole Sample Method:

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

Aliquot Method:

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

If sorting efficiency is greater than 95% (*i.e.*, no more than 5% of the organisms in the original sample are missed), then no action is required. Sorting efficiencies below 95% initiate continuous monitoring of the underperforming technician. Failure to achieve 95% sorting efficiency initiates re-sorting of all samples previously sorted by that technician. Organisms found during re-sort should be included in the results from the sample. The calculated sorting efficiency is recorded on the sorting form for each sample that is re-sorted. The laboratory responsible for sorting should retain sample debris left after sorting until cleared for disposal. The debris should be properly labeled and preserved with 70% ethanol.

## Taxonomic Analysis

The goal of taxonomic analysis is to identify accurately all organisms present in each sample to species level (or the lowest possible taxonomic level) and provide an accurate count of the organisms in each identified taxon.

Because of difficulties in the taxonomy and the lack of taxonomic expertise, exceptions to the goal of species-level identification are often established for a few groups of organisms. Examples are: Kinorhynchs are often only identified to phylum Kinorhyncha; in saline waters, Oligochaete annelids are often identified only to class Oligochaeta; Hirudinean annelids are often identified only to class Hirudinea; Podocopid ostracods are identified only to order Podocopida; and Harpacticoid copepods are identified only to order Harpacticoida.

Data for organisms that are incidental contaminants are not included in data analysis and should not be counted or included in project data. They are included as notes on the bench data sheets. For example, hard-bottom epifaunal organisms such as barnacles occur incidentally in samples collected immediately adjacent to hard structures such as piers in harbors; their counts should not be included in the project data.

The numbers of organisms reported for a sample should include all organisms alive at the time of collection. Care should be taken to not count any individual more than once. Inevitably, samples contain fragments of organisms. It is recommended that fragments of bilaterally symmetrical organisms should be identified and counted only if the fragment includes the anterior end of the organism. Only fragments of radially symmetrical organisms (*e.g.*, ophiuroids and anthozoans) containing the majority of the oral disk should be identified and counted. Care must be taken to avoid reporting empty mollusc shells or crustacean molts in the data.

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

Nomenclature and orthography should follow the usage in the SQO species list on the Sediment Quality Assessment Tools page of the Southern California Coastal Water Research Project (SCCWRP) website (http://www.sccwrp.org/view.php?id=565). This list represents a consensus for standard usage of taxon names in the data used to develop SQOs in southern California bays and estuaries and San Francisco Bay. These lists reflect the levels of identification used to calculate measures such as numbers of taxa and tolerance scores for benthic indices included in benthic line of evidence development. Compatibility of nomenclature is necessary to preserve compatibility of benthic index and assessment thresholds with those established as SQOs. Additional sources, such as Edition 5 of the Southern California Association of Marine Invertebrate Taxonomists' taxonomic listing, available at www.scamit.org, may also be useful because it lists synonyms that may have replaced names on the SQO species list.

Taxon (species) names in species abundance data tables (see data entry section that follows) follow special rules, and are standardized in spelling and form. Because the "species" field is one of the key fields for defining a unique record, exactitude is required. To minimize the problem of variants, standard spelling and formation for names is based on names in the SQO species list on the Sediment Quality Assessment Tools page of the SCCWRP website (http://www.sccwrp.org/view.php?id=565), and Edition 5 of the Southern California Association

of Marine Invertebrate Taxonomists' taxonomic listing, available at www.scamit.org. The name used to represent a taxon generally should be that listed in the species list and the species field should contain only genus and species names free of any punctuation, including periods, commas, and quotation marks. Descriptors such as "juvenile" or "fragment" are reserved for the comments field. If it is desired to separate adults and juveniles of a species, the number of juveniles can be carried in the comments field, but the abundance number should reflect the total number of animals of that species in that sample. The recommendations for levels of taxonomic resolution specified in the chapter providing guidance for SQO taxonomic analysis are also relevant. Samples with no organisms are recorded with the species name "No organisms present" and a blank (missing) or zero abundance to clearly indicate that the sample was collected but no organisms were present.

Temporary "in-house" provisional names are erected for specimens that a taxonomist considers to be distinctive but cannot match with an existing description. Provisional names should be resolved prior to data submission and analysis by assigning a valid name (binomen acceptable to the International Commission on Zoological Nomenclature (ICZN) or by documenting the characteristics of the provisional taxon on a voucher sheet that meets the Voucher Sheet standards of the Southern California Association of Marine Invertebrate Taxonomists (SCAMIT).

Taxonomists identifying and enumerating benthic macrofauna in samples should also be aware of and utilize "exclude" and "voucher" notations. The "exclude" field provides an aid to data analyses that involve calculating numbers of taxa by presenting the taxonomist's recommendation that the reported taxon be excluded from counts of the number of taxa in the sample. This is useful when the taxon in question is already included in another row. Specifically, the "exclude" annotation is employed when three conditions co-exist:

- The identification is not at the species-level (*e.g.*, Pleustidae or *Polydora* sp.)
- The reported taxon is represented in the sample by other members of its taxon, which have been identified at lower levels
- The taxonomist cannot determine if the specimen is distinct from other members of its taxon in the sample

Taxonomists should make this evaluation during sample analysis (*i.e.*, by an annotation on the bench sheet). It cannot be effectively applied after the fact, because there is no way of determining later whether the third criterion for use was met. An example would be recommending that organisms identified only as Spionidae be excluded because other organisms were identified as *Pseudopolydora paucibranchiata*, which is a spionid polychaete. The rationale for this is that, although the spionids should count when computing abundance, they should be excluded from calculations of numbers of taxa unless they are clearly not *P. paucibranchiata*.

Voucher counts document removal of specimens from a sample and this notation on the bench sheet is essential for integrity of the quality control and assessment process. Removal of

organisms for voucher collections without annotation confuses the resolution of discrepancies during quality control re-analysis, and leads to overstatement of error rates.

### *Quality Control*

The goal of taxonomic analysis for macrofaunal samples is species-level identification of all macrobenthic organisms collected, and an accurate count for each species. Establishing voucher collections and reanalysis of a subset are two types of recommended quality control activities. Quality assurance activities may include participation in regional or statewide taxonomic organizations (such as SCAMIT) or taxonomic workshops.

#### Voucher Collections

The purpose of a voucher collection is to provide good quality specimens exemplifying project taxonomists' usage of each name in the data. In cases where questions about nomenclature arise, or a portion or the entirety of a taxon is subsequently synonymized, examination of the vouchers may resolve uncertainties.

Each voucher container should contain an internal label bearing the complete taxon name, author, and date. Only glass containers are used for the storage of voucher material, unless specimens are inappropriate for wet storage. Within the voucher container, each specimen lot should be contained within a shell vial closed with a cotton stopper. Shell vials should have a minimum capacity of one-half dram. Specimens too large to be contained in shell vials may be stored in jars. Each voucher lot contains 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, and the identifying taxonomist's initials. Labels should be written in pencil or indelible ink on 100% rag-paper, poly-paper, or other paper suitable for permanent wet labels.

Subordinate to project voucher requirements, individual labs or taxonomists may remove a limited number of specimens for their own voucher collections. Any and all unique specimens are usually included in project, rather than individual, voucher collections.

#### Sample Re-analysis

Another approach recommended for providing data quality control is an assessment of the laboratory's accuracy by re-analysis of a subset of samples by independent taxonomists. Discrepancies between the original and quality control taxonomists are resolved by comparing results of the two sets of identifications.

Usually, about 10% of the samples processed by a laboratory are re-analyzed. Samples for re-identification are selected at random after identification is complete, ensuring that there is no prior knowledge of the identity of reanalysis samples. Re-identification should be conducted by taxonomists other than those who originally analyzed the samples. Quality control taxonomists should not have access to the original taxonomic analysis results.

After re-analysis is complete, quality control taxonomists are provided the original results, which are compared with the quality control re-analysis results, and all discrepancies are listed on a Discrepancy Report (Appendix C). Significant discrepancies in count (±5% of the original count) are usually resolved by a third count performed by the re-analytical lab. The original and quality control taxonomists then jointly identify causes and resolve discrepancies using a Discrepancy Resolution Report (Appendix C). This process may include consulting additional experts, if necessary. Once resolution and explanation of all discrepancies is complete, the Discrepancy Resolution Report, copies of both laboratories' bench sheets, and the Discrepancy Report are used to calculate the percent error of the original laboratory's analysis. It is recommended that percent error be calculated for three aspects of sample analysis: number of taxa discriminated ($\%Err_{\#\ Tax}$), total organism count ($\%Err_{\#\ Orgs}$), and identification accuracy ($\%Err_{ID}$) 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 consider only errors in the original analysis. The results are reported on an Infaunal Identification and Enumeration Accuracy Report (Appendix C).

Based upon the results of data quality assessment, a DQO of 10%, representing the maximum deviation from the "true" value, is recommended for number of taxa, total number of organisms, and identification accuracy. Each laboratory should strive to avoid exceeding this level of error.

## Data Entry

The taxonomic analysis results are usually stored in a species abundance data table that documents the numerical presence of all infaunal animals collected in each sample. Each row presents the abundance of a single species in a sample. The table contains as many rows as the sum of the number of taxa in all the samples. Details of the information usually included in the columns of a species abundance table are presented in Table 5.2.

**Table 5.2. Example table structure for species abundance data.**

| Field (Column) Name | Field Type | Field Required? | Description |
|---|---|---|---|
| StationID | Text | Yes | Station name |
| SampleID | Text | No | Internal laboratory sample identification |
| Replicate | Text | Yes | The sequential number of the grab |
| Sample Date | Date/Time | Yes | The sample collection date |
| Species | Text | Yes | The taxon name |
| Qualifier | Text | No | Abundance qualifier from an established list (*e.g.*, colonial organisms) |
| Abundance | Number | Yes | Number of individuals (0 for colonies) |
| Exclude | Yes/No | Yes | Flag to exclude when counting number of taxa |
| Lab Code | Text | Yes | Laboratory identification |
| Screen Size | Text | Yes | Sieve or screen size used to process samples - usually 1.0 or 0.5 |
| Screen Size Units | Text | Yes | Usually millimeters (mm) |
| Voucher | Number | No | Number of animals from this sample that were vouchered |
| Comments | Text | No | Comments |

Taxon (species) names in species abundance data tables follow special rules, and are standardized in spelling and form. Because the "species" field is a key field for defining unique records, exactitude is required. To minimize the problem of variants, standard spellings are on the SQO species list on the Sediment Quality Assessment Tools page of the SCCWRP website (http://www.sccwrp.org/view.php?id=565 ) and Edition 5 of the Southern California Association of Marine Invertebrate Taxonomists' taxonomic listing, available at www.scamit.org. Additional details about the contents of the species name field are presented in the taxonomic analysis section.

The exclude field provides an aid to data analysis for calculating numbers of taxa by documenting the taxonomist's recommendation that a taxon be excluded from taxon counts because it is already included in another row. Voucher notations document removal of specimens from a sample and are essential to the quality control and assessment process. Refer to the taxonomic analysis section of this chapter for more detailed information about the "exclude" and "voucher" notations.

## Data Analysis to Determine Benthic Invertebrate Community Condition

### *Introduction*

Research in California embayments has shown that the use of a combination of benthic indices provides a more accurate description of benthic invertebrate community condition than does the use of a single index (Ranasinghe *et al*. 2009). This chapter describes the steps necessary to calculate four benthic indices:

- Index of Biotic Integrity (IBI)
- Relative Benthic Index (RBI)
- Benthic Response Index (BRI)
- River Invertebrate Prediction and Classification System (RIVPACS)

Details about the history, background, and development of the indices and literature citations are provided in Ranasinghe *et al.* (2007). Each index assesses benthic condition of a sample in terms of one of four categories:

- Reference: A community that would occur at a reference site for that habitat
- Slight Disturbance: A community that may exhibit some indication of stress, but is within measurement variability of reference condition
- Moderate Disturbance: A community that exhibits clear evidence of physical, chemical, natural, or anthropogenic stress
- High Disturbance: A community exhibiting a high magnitude of stress

The steps necessary to determine the benthic community condition for a sample are:

1. Gathering data
2. Identifying the benthic habitat that was sampled in order to select appropriate benthic tools
3. Calculating four benthic indices and comparing benthic index to response ranges to determine condition categories
4. Integrating the individual index results to determine the benthic community condition classification (*i.e.*, the Benthic LOE).

Details of the steps to determine the benthic community condition category for a sample follow.

### *Data Preparation*

The raw data needed for the analyses include the abundance of each species (or lowest possible identification level or taxon) and station depth, latitude, and longitude. Each taxon should be identified to the appropriate level in keeping with the benthic macrofauna species list for the relevant habitat. When new taxa are encountered, the nomenclature and level of taxonomy should follow the species list, to the greatest extent possible.

### *Identification of Benthic Habitat Type*

Each benthic index must be calibrated to the natural assemblage characteristic of the sample site habitat. Six different assemblages are present in California embayments and the nature of the expected assemblage is determined by habitat factors, such as salinity, sediment grain size, bottom depth, latitude, and longitude (Ranasinghe *et al*. 2008). A key to identify the habitat type from these physical habitat factors and a table of dominant species, to verify that the assemblage corresponds with these factors, are provided in Appendix D.

### *Calculate Benthic Indices and Determine Condition Categories*

Each benthic index must be calibrated to the natural assemblage characteristic of the sample site habitat. Several different assemblages are present in California embayments. The nature of the expected assemblage is determined by habitat factors, such as salinity, grain size, bottom depth, latitude, and longitude. This document describes the calculation of benthic indices for the assemblages that are characteristic of two habitat types: Southern California Marine Bays and Polyhaline Central San Francisco Bay. Although the same four benthic indices are calculated for samples from each habitat, the metrics included in the RBI and IBI differ slightly, as do the species tolerance values for the BRI and habitat variables and lists of taxa for RIVPACS. Details of the metrics included in each habitat are presented in Table 5.3 and Table 5.10. Species lists that include habitat specific species tolerance values for the BRI and RIVPACS reference taxa are provided separately for each habitat.

### *Southern California Marine Bays*

Table 5.3 presents details of the metrics included for calculation of index values in Southern California Marine Bays. Instructions for calculating each of the indices and descriptions of the species list variables follow.

**Table 5.3. Benthic indicator metrics in southern California marine bays. Asterisks indicate metrics included in both the RBI and IBI.**

| Index | Metric | Use |
|---|---|---|
| IBI | Total number of taxa* | All taxa |
| | Number of mollusc taxa* | Molluscs |
| | *Notomastus* sp. abundance | *Notomastus* sp |
| | Abundance percentage of sensitive taxa | IBISensitive = S |
| RBI | Total number of taxa* | All taxa |
| | Number of mollusc taxa* | Molluscs |
| | Number of crustacean taxa | Crustaceans |
| | Number of crustacean individuals | Crustaceans |
| | Abundance of *Monocorophium insidiosum* | *Monocorophium insidiosum* |
| | Abundance of *Asthenothaerus diegensis* | *Asthenothaerus diegensis* |
| | Abundance of *Goniada littorea* | *Goniada littorea* |
| | Presence of *Capitella capitata* complex | *Capitella capitata* complex |
| | Presence of Oligochaeta | Oligochaeta |
| BRI | Abundance weighted average tolerance score | ToleranceScore |
| RIVPACS | Observed to expected (O/E) ratio for number of RIVPACS reference taxa | Instructions for calculating O/E Ratio using SAS Software (Appendix A) or the Utah State University web site (Appendix B). |

Index of Biotic Integrity (IBI) and IBI condition category

The IBI compares the values of four different metrics to the ranges expected under reference conditions. Each metric that is outside of the reference range increases the IBI score by one. Therefore, if all four metrics were inside the reference range, the score would be 0. Conversely, if all four were outside the reference range, the value would be 4.

The data needed to calculate the IBI are the total number of taxa, number of mollusc taxa, abundance of *Notomastus* sp., and number of sensitive taxa (Table 5.1). The total number of taxa, number of mollusc taxa, and abundance of *Notomastus* sp. can be obtained directly from the data. The list of sensitive species should be based on the species list for Southern California Marine Bays and the percentage of sensitive taxa present is calculated as:

**% sensitive taxa= (number of sensitive taxa/total number of taxa) * 100**

The value for each metric is then compared to a reference range for that metric (Table 5.4). The IBI score is set to zero before comparison to the reference range. For each metric that is out of the reference range (above or below), the IBI score goes up by one.

**Table 5.4. Reference ranges for IBI metrics in southern California marine bays.**

| Metric | Reference Range |
|---|---|
| Total Number of Taxa | 13 - 99 |
| Number of Mollusc Taxa | 2 - 25 |
| Abundance of *Notomastus sp.* | 0 - 59 |
| Percentage of Sensitive Taxa | 19 - 47.1 |

The IBI score is then compared to condition category response ranges (Table 5.5) in order to determine the IBI category and score.

**Table 5.5. IBI category response ranges for southern California marine bays.**

| IBI Score | Category | Category Score |
|---|---|---|
| 0 | Reference | 1 |
| 1 | Low Disturbance | 2 |
| 2 | Moderate Disturbance | 3 |
| 3 or 4 | High Disturbance | 4 |

Relative Benthic Index (RBI) and RBI Condition Category

The RBI is the weighted sum of: 1) four community metrics related to biodiversity (total number of taxa, number of crustacean taxa, abundance of crustacean individuals, and number of mollusc taxa); 2) abundances of three positive indicator taxa; and 3) the presence of two negative indicator species.

The data needed to calculate the RBI are: total number of taxa, number of mollusc taxa, number of crustacean taxa, number of crustacean individuals, number of individuals of *Monocorophium insidiosum*, *Asthenothaerus diegensis*, and *Goniada littorea*, and the presence of *Capitella capitata* complex and Oligochaeta.

The first step is to normalize the values for the benthic community metrics relative to maxima for the data used to develop the RBI for the Southern California Marine Bays habitat, to produce values relative to the maxima that are referred to as scaled values. The scaled value calculations use the following formulae:

Total number of taxa/99

Number of mollusc taxa/28

Number of crustacean taxa/29

Abundance of Crustacea/1693

The next step is to calculate the Taxa Richness Weighted Value (TWV) from the scaled values by the equation:

> TWV = Scaled total number of taxa + Scaled number of mollusc taxa + Scaled number of crustacean taxa + (0.25 * Scaled abundance of Crustacea)

Next, the value for the two negative indicator taxa (NIT) is calculated. The two negative indicator taxa are *Capitella capitata* complex and Oligochaeta. For each of these taxa that are present, in any abundance whatsoever, the NIT is decreased by 0.1. Therefore, if neither were found the NIT = 0, if both are found the NIT = -0.2.

The next step is to calculate the value for the three positive indicator taxa (PIT). The positive indicator taxa are *Monocorophium insidiosum*, *Asthenothaerus diegensis*, and *Goniada littorea.* First, the PIT value is calculated for each species using the following equations:

$$\frac{\sqrt[4]{\textit{Monocorophium insidiosum}\ \text{abundance}}}{\sqrt[4]{473}}$$

$$\frac{\sqrt[4]{\textit{Asthenothaerus diegensis}\ \text{abundance}}}{\sqrt[4]{27}}$$

$$\frac{\sqrt[4]{\textit{Goniada littorea}\ \text{abundance}}}{\sqrt[4]{15}}$$

The three species PIT values are then summed to calculate the PIT value for the sample. If none of the three species is present, then the sample PIT = 0.

The next step is to calculate the Raw RBI:

Raw RBI = TWV + NIT + (2 * PIT)

The final calculation is for the RBI Score, normalizing the Raw RBI by the minimum and maximum Raw RBI values in the index development data:

RBI Score = (Raw RBI - 0.03)/4.69

The last step in the RBI process is to compare the RBI Score to a set of response ranges to determine the RBI category (Table 5.6).

**Table 5.6. RBI category response ranges for southern California marine bays.**

| RBI Score | Category | Category Score |
|---|---|---|
| >0.27 | Reference | 1 |
| >0.16 to ≤0.27 | Low Disturbance | 2 |
| >0.08 to ≤0.16 | Moderate Disturbance | 3 |
| ≤0.08 | High Disturbance | 4 |

Benthic Response Index (BRI) and BRI Condition Category

The BRI is the abundance weighted pollution tolerance score of the organisms present in a benthic sample. The higher the BRI score, the more degraded the benthic community represented by the sample.

Two types of data are needed to calculate the BRI, the abundance of each species and its pollution tolerance score, P. P values are available for most species present in the assemblage. Only species for which P values are available are used in the BRI calculations. P values should be obtained for the appropriate habitat and from the most up-to-date list available.

The first step in the BRI calculation is to compute the $4^{th}$ root of the abundance of each taxon in the sample for which P values are available. The next step is to multiply the $4^{th}$ root abundance value by the P value, for each taxon.

Next, separately sum all of the $4^{th}$ roots of the abundances and all of the products of the $4^{th}$ roots of abundance and P values. Taxa that lack P values are not included in either sum.

The next step is to calculate the BRI score as:

$$\frac{\sum\left(\sqrt[4]{Abundance}\right)\times P}{\sum \sqrt[4]{Abundance}}$$

The last step is to compare the BRI score to BRI response ranges in Table 5.7 to determine the BRI category and category score.

**Table 5.7. BRI category response ranges for southern California marine bays.**

| BRI Score | Category | Category Score |
|---|---|---|
| <39.96 | Reference | 1 |
| ≥39.96 to <49.15 | Low Disturbance | 2 |
| ≥49.15 to <73.27 | Moderate Disturbance | 3 |
| ≥73.27 | High Disturbance | 4 |

River Invertebrate Prediction and Classification System (RIVPACS) Index and RIVPACS Condition Category

The RIVPACS index calculates the number of reference taxa present in the test sample (observed or "O") and compares it to the number expected to be present ("E") in a reference sample from the same habitat. Calculation of the RIVPACS score is a three-step process. The first step consists of determining the probability of the test sample belonging to twelve Southern California Marine Bays reference sample groups. This determination is based on the sampling station's bottom depth, latitude, and longitude, using a complex linear discriminant function.

The second step is determining, for each sample, the identity and expected number of reference species, based on the probabilities of group membership calculated in Step 1 and the distribution of reference species in each group. In the final step, the number of reference species observed in the sample is counted, the observed/expected (O/E) RIVPACS score calculated and compared to the response ranges in Table 5.8 to determine the RIVPACS category and category score.

**Table 5.8. RIVPACS category response ranges for southern California marine bays.**

| RIVPACS Score | Category | Category Score |
|---|---|---|
| >0.90 - <1.10 | Reference | 1 |
| >0.74 - ≤0.90 or ≥1.10 - <1.26 | Low Disturbance | 2 |
| >0.32 - ≤0.74 or ≥1.26 | Moderate Disturbance | 3 |
| ≤0.32 | High Disturbance | 4 |

Because of the complexity of the RIVPACS calculations, computer programs are used to determine the O/E values. Detailed instructions for calculating RIVPACS O/E values by two computer programs are provided in Appendices A and B. Appendix A contains instructions for calculating RIVPACS O/E values using the Statistical Analysis System (SAS), while Appendix B contains instructions for calculating these values using the website at Utah State University's Western Center for Monitoring and Assessment of Freshwater Ecosystems. The SAS programs calculate RIVPACS O/E values and condition categories, but require availability of the SAS software. The Utah State University website is freely available to calculate RIVPACS O/E values, but data requirements are rigid and application of response ranges to determine condition categories is a separate procedure.

Species List Contents

The Southern California Marine Bays species list is provided in a spreadsheet that can be accessed from the Sediment Quality Assessment Tools page of the SCCWRP web site (http://www.sccwrp.org/view.php?id=565). The contents of each column in the spreadsheet are described in Table 5.9.

**Table 5.9. Southern California marine bays species list contents.**

| Column | Header | Contents |
|---|---|---|
| 1 | TaxonName | Taxon name |
| 2 | Phylum | Taxonomic phylum |
| 3 | Class | Taxonomic class |
| 4 | Order | Taxonomic order |
| 5 | Family | Taxonomic family |
| 6 | IBISensitive | When present, "S" indicates a taxon considered sensitive for calculation of the SoCal IBI |
| 7 | Mollusc | When present, "Mollusc" indicates molluscan taxa for RBI and IBI calculations |
| 8 | Crustacean | When present, "Crustacean" indicates crustacean taxa for RBI calculations |
| 9 | Tolerance Score | When present, values are tolerance scores for BRI calculation |
| 10 | RivColHead | When present, in the abundance data file submitted for RIVPACS calculations to the Utah State University web site, this exact text is used as the column header for abundance data for this taxon |
| 11 | RivColNo | When present, in the abundance data file submitted for RIVPACS calculations to the Utah State University web site, this is the column number containing abundances for this taxon. |
| 12 | SpeciesLevel | When present, "Drop" in this column indicates that abundances of this taxon are included in index calculations, but it is not included for counting numbers of taxa because lower taxonomic level entries in this taxon are also present. |

Polyhaline Central San Francisco Bay

Table 5.10 presents details of the metrics included for calculation of index values in Polyhaline Central San Francisco Bay. Instructions for calculating each of the indices and descriptions of the species list variables follow.

**Table 5.10. Benthic indicator metrics in polyhaline central San Francisco Bay. Asterisks indicate metrics included in both the RBI and IBI.**

| Index | Metric | Use |
|---|---|---|
| **IBI** | Total number of taxa* | All taxa |
| | Number of amphipod taxa | Amphipods |
| | Total abundance | All taxa |
| | Abundance of *Capitella capitata* complex | *Capitella capitata* Cmplx |
| **RBI** | Total number of taxa* | All taxa |
| | Number of mollusc taxa | Molluscs |
| | Number of crustacean taxa | Crustaceans |
| | Number of crustacean individuals | Crustaceans |
| | Abundance of *Sinocorophium heteroceratum* | *Sinocorophium heteroceratum* |
| | Abundance of *Rochefortia* spp. | *Rochefortia* spp. |
| | Abundance of *Prionospio (Minuspio) lighti* | *Prionospio (Minuspio) lighti* |
| | Presence of *Capitella capitata* complex | *Capitella capitata* Cmplx |
| | Presence of Oligochaeta | Oligochaeta |
| **BRI** | Abundance weighted average tolerance score | ToleranceScore |
| **RIVPACS** | Observed to expected ratio for number of RIVPACS reference taxa. | Instructions for calculating O/E Ratio using SAS Software (Appendix A) or the Utah State University web site (Appendix B). |

Index of Biotic Integrity (IBI) and IBI Condition Category

The IBI compares the values of four different metrics to the ranges expected under reference conditions. Each metric that is outside of the reference range increases the IBI score by one. Therefore, if all four metrics were inside the reference range, the score would be 0. Conversely, if all four were outside the reference range, the value would be 4.

The data needed to calculate the IBI are the total number of taxa, number of amphipod taxa, total abundance, and abundance of *Capitella capitata* complex (Table 5.10), which can be obtained directly from the data.

The value for each metric is then compared to a reference range for that metric (Table 5.11). The IBI score is set to zero before comparison to the reference ranges. For each metric that is out of the reference range (above or below), the IBI score goes up by one.

**Table 5.11. Reference ranges for IBI metrics in polyhaline central San Francisco Bay.**

| Metric | Reference Range |
|---|---|
| Total Number of Taxa | 21 - 66 |
| Number of Amphipod Taxa | 2 - 11 |
| Total Abundance | 97 - 2931 |
| Abundance of *Capitella capitata* complex | 0 - 13 |

The IBI score is then compared to condition category response ranges (Table 5.12) in order to determine the IBI category and score.

**Table 5.12. IBI category response ranges for polyhaline central San Francisco Bay.**

| IBI Score | Category | Category Score |
|---|---|---|
| 0 or 1 | Reference | 1 |
| 2 | Low Disturbance | 2 |
| 3 | Moderate Disturbance | 3 |
| 4 | High Disturbance | 4 |

Relative Benthic Index (RBI) and RBI Condition Category

The RBI is the weighted sum of: 1) four community metrics related to biodiversity (total number of taxa, number of crustacean taxa, abundance of crustacean individuals, and number of mollusc taxa); 2) abundances of three positive indicator taxa; and 3) the presence of two negative indicator species.

The data needed to calculate the RBI are: total number of taxa, number of mollusc taxa, number of crustacean taxa, number of crustacean individuals, number of individuals of *Sinocorophium heteroceratum*, the genus *Rochefortia*, and *Prionospio (Minuspio) lighti*, and the presence of *Capitella capitata* complex and Oligochaeta.

The first step is to normalize the values for the benthic community metrics relative to maxima for the data used to develop the RBI for the Polyhaline Central San Francisco Bay habitat, to produce values relative to the maxima that are referred to as scaled values. The scaled value calculations use the following formulae:

Total number of taxa/55

Number of mollusc taxa/13

Number of crustacean taxa/17

Abundance of Crustacea/17237

The next step is to calculate the TWV from the scaled values by the equation:

TWV = Scaled total number of taxa + Scaled number of mollusc taxa + Scaled number of crustacean taxa + (0.25 * Scaled abundance of Crustacea)

Next, the value for the two negative indicator taxa (NIT) is calculated. The two negative indicator taxa are *Capitella capitata* complex and Oligochaeta. For each of these taxa that are present, in any abundance whatsoever, the NIT is decreased by 0.1. Therefore, if neither were found the NIT = 0, if both are found the NIT = -0.2.

The next step is to calculate the value for the three PIT. The positive indicator taxa are *Sinocorophium heteroceratum*, *Rochefortia* spp, and *Prionospio (Minuspio) lighti.* First, the PIT value is calculated for each species using the following equations:

$$\frac{\sqrt[4]{Sinocorophium\ heteroceratum\ \text{abundance}}}{\sqrt[4]{1878}}$$

$$\frac{\sqrt[4]{Rochefortia\ \text{spp. abundance}}}{\sqrt[4]{105}}$$

$$\frac{\sqrt[4]{Prionspio\ (Minuspio)\ lighti\ \text{abundance}}}{\sqrt[4]{17}}$$

The three species PIT values are then summed to calculate the PIT value for the sample. If none of the three species is present, then the sample PIT = 0.

The next step is to calculate the Raw RBI:

Raw RBI = TWV + NIT + (2 * PIT)

The final calculation is for the RBI Score, normalizing the Raw RBI by the minimum and maximum Raw RBI values in the index development data:

RBI Score = (Raw RBI - 0.00)/6.88

The last step in the RBI process is to compare the RBI Score to a set of response ranges to determine the RBI category (Table 5.13).

**Table 5.13. RBI category response ranges for polyhaline central San Francisco Bay.**

| RBI Score | Category | Category Score |
|---|---|---|
| >0.43 | Reference | 1 |
| >0.29 - ≤0.43 | Low Disturbance | 2 |
| >0.19 - ≤0.29 | Moderate Disturbance | 3 |
| ≤0.19 | High Disturbance | 4 |

Benthic Response Index (BRI) and BRI Condition Category

The BRI is the abundance weighted pollution tolerance score of the organisms present in a benthic sample. The higher the BRI score, the more degraded the benthic community represented by the sample.

Two types of data are needed to calculate the BRI, the abundance of each species and its pollution tolerance score, P. P values are available for most species present in the assemblage. Only species for which P values are available are used in the BRI calculations. P values should be obtained for the appropriate habitat and from the most up-to-date list available.

The first step in the BRI calculation is to compute the $4^{th}$ root of the abundance of each taxon in the sample for which P values are available. The next step is to multiply the $4^{th}$ root abundance value by the P value, for each taxon.

Next, separately sum all of the $4^{th}$ roots of the abundances and all of the products of the $4^{th}$ roots of abundance and P values. Taxa that lack P values are not included in either sum.

The next step is to calculate the BRI score as:

$$\frac{\sum\left(\sqrt[4]{Abundance}\right)\times P}{\sum \sqrt[4]{Abundance}}$$

The last step is to compare the BRI score to BRI response ranges in Table 5.14 to determine the BRI category and category score.

**Table 5.14. BRI category response ranges for polyhaline central San Francisco Bay.**

| BRI Score | Category | Category Score |
|---|---|---|
| < 22.28 | Reference | 1 |
| ≥ 22.28 to <33.38 | Low Disturbance | 2 |
| ≥ 33.38 to <82.09 | Moderate Disturbance | 3 |
| ≥ 82.09 | High Disturbance | 4 |

River Invertebrate Prediction and Classification System (RIVPACS) Index and RIVPACS Condition Category

The RIVPACS index calculates the number of reference taxa present in the test sample (observed or "O") and compares it to the number expected to be present ("E") in a reference sample from the same habitat. Calculation of the RIVPACS score is a three- step process. The first step consists of determining the probability of the test sample belonging to four Polyhaline Central San Francisco Bay reference sample groups. This determination is based on the sampling station's bottom depth and longitude, using a complex linear discriminant function.

The second step is determining, for each sample, the identity and expected number of reference species, based on the probabilities of group membership calculated in Step 1 and the distribution of reference species in each group. In the final step, the number of reference species observed in the sample is counted, the O/E RIVPACS score calculated and compared to the response ranges in Table 5.15 to determine the RIVPACS category and category score.

**Table 5.15. RIVPACS category response ranges for polyhaline central San Francisco Bay.**

| RIVPACS Score | Category | Category Score |
|---|---|---|
| >0.68 - <1.32 | Reference | 1 |
| >0.32 - ≤0.68 or ≥1.32 - <1.68 | Low Disturbance | 2 |
| >0.15 - ≤0.32 or ≥1.68 | Moderate Disturbance | 3 |
| ≤0.15 | High Disturbance | 4 |

Because of the complexity of the RIVPACS calculations, computer programs are used to determine the O/E values. Detailed instructions for calculating RIVPACS O/E values by two alternate computer programs are provided in Appendices A and B. Appendix A contains instructions for calculating RIVPACS O/E values using the SAS, while Appendix B contains instructions for calculating these values using the website at Utah State University's Western Center for Monitoring and Assessment of Freshwater Ecosystems. The SAS programs calculate RIVPACS O/E values and condition categories, but require availability of the SAS software. The Utah State University website is freely available to calculate RIVPACS O/E values, but data requirements are rigid and application of thresholds to determine condition categories is a separate procedure.

Species List

The Polyhaline Central San Francisco Bay species list is provided on a spreadsheet that can be accessed from the Sediment Quality Assessment Tools page of the SCCWRP web site (http://www.sccwrp.org/view.php?id=565). The contents of each column on the spreadsheet are described in Table 5.16.

**Table 5.16. Polyhaline central San Francisco Bay species list contents.**

| Column | Header | Contents |
|---|---|---|
| 1 | TaxonName | Taxon name |
| 2 | Phylum | Taxonomic phylum |
| 3 | Class | Taxonomic class |
| 4 | Order | Taxonomic order |
| 5 | Family | Taxonomic family |
| 6 | Mollusc | When present, "Mollusc" indicates molluscan taxa for RBI calculations |
| 7 | Crustacean | When present, "Crustacean" indicates crustacean taxa for RBI calculations |
| 8 | Amphipod | When present, "Amphipod" indicates amphipod taxa for IBI calculations |
| 9 | Tolerance Score | When present, values are tolerance scores for BRI calculation |
| 10 | RivColHead | When present, in the abundance data file submitted for RIVPACS calculations to the Utah State University web site, this exact text is used as the column header for abundance data for this taxon |
| 11 | RivColNo | When present, in the abundance data file submitted for RIVPACS calculations to the Utah State University web site, this is the column number containing abundances for this taxon. |
| 12 | SpeciesLevel | When present, "Drop" in this column indicates that abundances of this taxon are included in index calculations, but it is not included for counting numbers of taxa because lower taxonomic level entries in this taxon are also present. |

### *Integration of Benthic Index Category Scores*

The final Benthic LOE category is derived by integrating all four benthic index category scores. The procedure is the same for samples from Southern California Marine Bays and samples from Polyhaline Central San Francisco Bay. Integration is accomplished by calculating the median of the four individual index category scores. If the median falls between two adjacent categories, the value is rounded up to the next highest integer. The Benthic LOE category names (and corresponding scores) are the same as those described for the individual indices.

## Example of Benthic Community Line of Evidence Calculation

For the Benthic LOE, the steps involved are gathering the data, calculating benthic community indices, comparing the index values to response ranges, and integrating the individual index results into a single Benthic LOE. While the general process of calculating the indices is similar between habitat types, the details may differ. The following example calculations are for the Southern California Marine Bays habitat. Most of the benthic index calculations can be made with a hand calculator, but it is simpler to use a spreadsheet program, such as Excel.

### *Data Preparation*

A sample data set is shown in Table 5.17. This table presents species abundances for all the benthic organisms found at the station. Each species is designated as sensitive or not, based on a list of sensitive species for the habitat, and identified as to whether it is a mollusc, crustacean, or neither.

**Table 5.17. Example benthic community data set.**

| Species Name | Abundance | Sensitive | Mollusc | Crustacean |
|---|---|---|---|---|
| *Acteocina inculta* | 296 | Yes | Yes | No |
| *Ampithoe valida* | 9 | Yes | No | Yes |
| *Capitella capitata* Cmplx | 764 | No | No | No |
| Chironomidae | 17 | No | No | No |
| *Dipolydora* sp | 73 | No | No | No |
| *Exogone lourei* | 5 | Yes | No | No |
| *Geukensia demissa* | 1 | No | Yes | No |
| *Grandidierella japonica* | 1116 | No | No | Yes |
| Harpacticoida | 1 | No | No | Yes |
| *Hemigrapsus oregonensis* | 1 | No | No | Yes |
| Lineidae | 1 | No | No | No |
| *Marphysa angelensis* | 9 | No | No | No |
| *Marphysa stylobranchiata* | 2 | No | No | No |
| *Mayerella acanthopoda* | 1 | No | No | Yes |
| *Mediomastus* sp | 2 | No | No | No |

**Table 5.17 Continued.**

| Species Name | Abundance | Sensitive | Mollusc | Crustacean |
|---|---|---|---|---|
| *Monocorophium insidiosum* | 3 | Yes | No | Yes |
| *Musculista senhousia* | 27 | No | Yes | No |
| Oligochaeta | 1584 | No | No | No |
| Podocopida | 1 | No | No | Yes |
| *Polydora nuchalis* | 73 | No | No | No |
| *Protothaca* sp | 1 | No | Yes | No |
| *Pseudopolydora paucibranchiata* | 60 | No | No | No |
| *Streblospio benedicti* | 1459 | No | No | No |
| *Tagelus subteres* | 4 | Yes | Yes | No |
| *Tryonia* sp | 2 | No | Yes | No |
| *Tubulanus* sp | 1 | No | No | No |
| *Turbellaria* | 1 | No | No | No |

### *Index of Biotic Integrity (IBI)*

The specific data needed to calculate the IBI are the total number of taxa, number of mollusc taxa, abundance of *Notomastus* sp., and number of sensitive taxa. The sensitive species list should be from the list specific to the station's habitat.

The IBI metric values for the sample data set are presented in Table 5.18. There were 27 different taxa represented in the sample, 6 of which were molluscs. There were no occurrences of the polychaete, *Notomastus* sp. Finally, there were 5 sensitive species in the sample, which represents 18.5% of the taxa, based on the following:

**% sensitive taxa= (number of sensitive taxa/total number of taxa) * 100**

**Table 5.18. IBI metrics for sample data set.**

| Metric | Value |
|---|---|
| Total Number of Taxa | 27 |
| Number of Mollusc Taxa | 6 |
| Abundance of *Notomastus* sp. | 0 |
| Percentage of Sensitive Taxa | 18.5 |

Once the IBI metrics have been calculated, the next step is to compare the values for each of the metrics to a reference range for that specific metric (Table 5.19). The IBI score is set to zero before comparison to the reference ranges. For each metric that is out of the reference range (above or below), the IBI score goes up by one.

For the sample data set, the total number of taxa, number of mollusc taxa and abundance of *Notomastus* sp. all fell within their reference ranges and therefore did not cause the IBI score to rise. However, the percentage of sensitive taxa was below the reference range and therefore caused the IBI score to rise by one. The final IBI score for this data set is thus 1.

**Table 5.19. Reference ranges for IBI metrics.**

| Metric | Reference Range |
|---|---|
| Total Number of Taxa | 13 - 99 |
| Number of Mollusc Taxa | 2 - 25 |
| Abundance of *Notomastus* sp. | 0 - 59 |
| Percentage of Sensitive Taxa | 19 - 47.1 |

The final step is to compare the IBI score to the category response ranges (Table 5.20) in order to determine the IBI category and score. For the example, the IBI score of 1 corresponds to the Low Disturbance category with a category score of 2.

**Table 5.20. IBI category response ranges.**

| IBI Score | Category | Category Score |
|---|---|---|
| 0 | Reference | 1 |
| 1 | Low Disturbance | 2 |
| 2 | Moderate Disturbance | 3 |
| 3 or 4 | High Disturbance | 4 |

### *Relative Benthic Index (RBI)*

The RBI is the weighted sum of: 1) several community metrics, 2) the abundances of three positive indicator species, and 3) the presence of two negative indicator species.

The first step is to normalize the values for the benthic community metrics relative to the test sample habitat type. In the case of this example the data come from the Southern California Marine Bays habitat. These values are referred to as the scaled values. The calculations use the following four equations:

Total number of taxa/99

Number of mollusc taxa/28

Number of crustacean taxa/29

Abundance of Crustacea/1693

The results of these calculations using the sample data set are shown in Table 5.21.

**Table 5.21. Scaled RBI Metric Values.**

| RBI Metric | Raw | Scaled |
|---|---|---|
| Total number of taxa | 27 | 0.272727 |
| Number of Mollusc taxa | 6 | 0.214286 |
| Number of Crustacean taxa | 7 | 0.241379 |
| Abundance of Crustacea | 1132 | 0.668636 |

The next step is to calculate the TWV. This is calculated using the following:

TWV = Scaled total number of taxa + Scaled number of mollusc taxa + Scaled number of crustacean taxa + (0.25 * Scaled abundance of Crustacea)

For the sample data set the TWV= 0.89555.

Next, the value for the two NIT is calculated. The two negative indicator taxa are *Capitella capitata* complex and Oligochaeta. For each of these taxa that are present, in any abundance whatsoever, the NIT is decreased by 0.1. Therefore, if neither were found the NIT = 0, if both are found the NIT = -0.2. For our example data, both taxa were present, so the NIT = -0.2.

The next step is to calculate the value for the three PIT. The positive indicator taxa are *Monocorophium insidiosum*, *Asthenothaerus diegensis*, and *Goniada littorea.* First, the PIT value is calculated for each species using the following equations:

$$\frac{\sqrt[4]{\textit{Monocorophium insidiosum}\text{ abundance}}}{\sqrt[4]{473}}$$

$$\frac{\sqrt[4]{\textit{Asthenothaerus diegensis}\text{ abundance}}}{\sqrt[4]{27}}$$

$$\frac{\sqrt[4]{\textit{Goniada littorea}\text{ abundance}}}{\sqrt[4]{15}}$$

The three species PIT values are then summed to calculate the PIT value for the sample. If none of the three species is present, then the sample PIT = 0. For the example data, only *M. insidiosum* was present and the result of its calculation was 0.282205, which in the absence of the other species is also the PIT value.

The next step is to calculate the Raw RBI:

Raw RBI = TWV + NIT + (2 * PIT)

For the sample data set:

Raw RBI = 0.89555 + (-0.2) + (2 * 0.282205) = 1.25996

The final calculation is for the RBI Score:

RBI Score = (Raw RBI - 0.03)/4.69

For the sample data set:

RBI Score = (1.25996 - 0.03)/4.69 = 0.26

**Table 5.22. RBI category response ranges.**

| RBI Score | Category | Category Score |
|---|---|---|
| >0.27 | Reference | 1 |
| >0.16 - ≤0.27 | Low Disturbance | 2 |
| >0.08 - ≤0.16 | Moderate Disturbance | 3 |
| ≤0.08 | High Disturbance | 4 |

### *Benthic Response Index (BRI)*

The BRI is the abundance weighted pollution tolerance score of the organisms present in a given benthic community sample. The higher the BRI score, the more degraded the benthic community present in the sample.

The first step in the BRI calculation is to compute the $4^{th}$ root of the abundance of each taxon in the sample for which pollution tolerance (P) values are available. For the sample data set, the calculated values are found in Table 5.23. The next step is to multiply the $4^{th}$ root abundance value by the P value, for each taxon (Table 5.23).

Next, separately sum all of the $4^{th}$ roots of the abundances and all of the products of the $4^{th}$ roots of abundance and P values (Table 5.23). Any taxa that lack P values are not included in either sum.

The next step is to calculate the BRI score as:

$$\frac{\sum\left(\sqrt[4]{Abundance}\right)\times P}{\sum \sqrt[4]{Abundance}}$$

For the sample data set, the BRI score is 82.56.

The last step is to compare the BRI score to BRI response range values in Table 5.24 to determine the BRI category and category score. For the example, the BRI corresponds to the High Disturbance category, with a category score of 4.

**Table 5.23. BRI component calculations for the sample data set. na = pollution tolerance (P) value not available for that taxon.**

| Taxon Name | Abundance | P | Abundance 4th root | Abundance 4th root * P |
|---|---|---|---|---|
| *Acteocina inculta* | 296 | 110.15 | 4.1478 | 456.88 |
| *Ampithoe valida* | 9 | 90.96 | 1.7321 | 157.56 |
| *Capitella capitata* Cmplx | 764 | 130.84 | 5.2574 | 687.90 |
| Chironomidae | 17 | 138.87 | 2.0305 | 281.99 |
| *Dipolydora sp* | 73 | 56.56 | 2.9230 | 165.33 |
| *Exogone lourei* | 5 | 41.86 | 1.4953 | 62.59 |
| *Geukensia demissa* | 1 | na | na | na |
| *Grandidierella japonica* | 1116 | 105.98 | 5.7798 | 612.57 |
| Harpacticoida | 1 | 32.91 | 1 | 32.91 |
| *Hemigrapsus oregonensis* | 1 | 60.70 | 1 | 60.70 |
| Lineidae | 1 | 3.96 | 1 | 3.96 |
| *Marphysa angelensis* | 9 | 97.82 | 1.7321 | 169.43 |
| *Marphysa stylobranchiata* | 2 | 94.27 | 1.1892 | 112.10 |
| *Mayerella acanthopoda* | 1 | 22.26 | 1 | 22.26 |
| *Mediomastus sp* | 2 | 57.84 | 1.1892 | 68.78 |
| *Monocorophium insidiosum* | 3 | 103.42 | 1.3161 | 136.11 |
| *Musculista senhousia* | 27 | 68.05 | 2.2795 | 155.12 |
| Oligochaeta | 1584 | 69.96 | 6.3087 | 441.35 |
| Podocopida | 1 | na | na | na |
| *Polydora nuchalis* | 73 | 108.42 | 2.9230 | 316.91 |
| *Protothaca sp* | 1 | 55.94 | 1 | 55.94 |
| *Pseudopolydora paucibranchiata* | 60 | 81.68 | 2.7832 | 227.34 |
| *Streblospio benedicti* | 1459 | 61.83 | 6.1804 | 382.11 |
| *Tagelus subteres* | 4 | 37.28 | 1.4142 | 52.73 |
| *Tryonia sp* | 2 | 127.95 | 1.1892 | 152.16 |
| *Tubulanus sp* | 1 | 0.61 | 1 | 0.61 |
| Turbellaria | 1 | 44.95 | 1 | 44.95 |
| Sum | | | 58.8708 | 4860.23 |

The last step in the RBI process is to compare the RBI Score to a set of response ranges to determine the RBI category (Table 5.22). For the example, the RBI score falls into the Low Disturbance category, with a category score of 2.

**Table 5.24. BRI category response ranges and category scores.**

| BRI Score | Category | Category Score |
|---|---|---|
| <39.96 | Reference | 1 |
| ≥39.96 - <49.15 | Low Disturbance | 2 |
| ≥49.15 - <73.27 | Moderate Disturbance | 3 |
| ≥73.27 | High Disturbance | 4 |

### *River Invertebrate Prediction and Classification System (RIVPACS)*

The RIVPACS index calculates the number of reference taxa present in the test sample (observed or "O") and compares it to the number expected to be present ("E") in a reference sample from the same habitat. Calculation of the RIVPACS score is a three-step process. The first step consists of determining the reference station group within the Southern California Marine Bays habitat to which the station belongs. This determination is made based on the station's bottom depth, latitude, and longitude. These three parameters are used with a discriminant function to estimate the probability that the station belongs to each reference station group.

The expected number of reference site species for the station is calculated in the second step. Since each reference station group may contain a different number of reference species, the expected number of reference species for the test station example is determined using the probabilities of reference group membership calculated in step 1. The expected number of reference site species (E) for the sample data set is 4.447.

The final step consists of calculating the RIVPACS score (O/E). The number of reference site species present in the sample data set (O) is five. The RIVPACS score is therefore 1.124 (5/4.447).

The score is then compared to the response ranges in Table 5.25 to determine the RIVPACS category and category score. For the example, the RIVPACS score corresponds to the Low Disturbance category, with a category score of 2.

**Table 5.25. RIVPACS category response ranges and category scores.**

| RIVPACS Score | Category | Category Score |
|---|---|---|
| > 0.90 - < 1.10 | Reference | 1 |
| > 0.74 - ≤ 0.90 or ≥ 1.10 - < 1.26 | Low Disturbance | 2 |
| > 0.32 to ≤ 0.74 or ≥ 1.26 | Moderate Disturbance | 3 |
| ≤ 0.32 | High Disturbance | 4 |

### *Integration of Benthic Community Indices*

The Benthic LOE category is based on the integration of the four benthic index category scores. The integration is accomplished by calculating the median of the four individual index category scores. If the median falls between two adjacent categories, the value is rounded up. For the sample data set, the index category scores were 2, 2, 2 and 4 for the IBI, RBI, RIVPACS, and BRI, respectively. The median for those values is 2. Therefore, the Benthic LOE for the example is Low Disturbance.

# CHAPTER 6: INTEGRATING THE LINES OF EVIDENCE, INTERPRETING RESULTS, AND NEXT STEPS

## Objectives

Previous chapters in this manual describe methods for estuarine and marine sediment sampling and analyses of chemistry, toxicity, and benthic community in order to determine three lines of evidence describing sediment quality. The objective of this chapter is to describe how the lines of evidence are integrated to arrive at a condition assessment category for each sampling station and provide suggestions for interpreting the results.

## Scope

There are numerous approaches for integrating multiple lines of evidence (MLOE) data in a sediment quality triad assessment, but most rely at least partially on best professional judgment, which can be problematic in application to large data sets or in a regulatory setting where the assessment protocol needs to be transparent and consistently reproducible. The integration approach described in this manual was developed for the California Sediment Quality Objectives (CASQO) program and consists of a standardized set of LOE relationships and final station assessments. The station assessments consist of six categories that describe likelihood and severity of direct effects from sediment contamination.

This assessment has two key limitations. First, it is relevant only for assessing impacts on aquatic life (*e.g.*, benthic community) from direct exposure to sediment contaminants; it does not represent impacts to human health or wildlife resulting from indirect exposures as a result of the bioaccumulation and/or biomagnification of contaminants in fish and shellfish. Second, the assessment does not identify the specific chemicals causing the impacts.

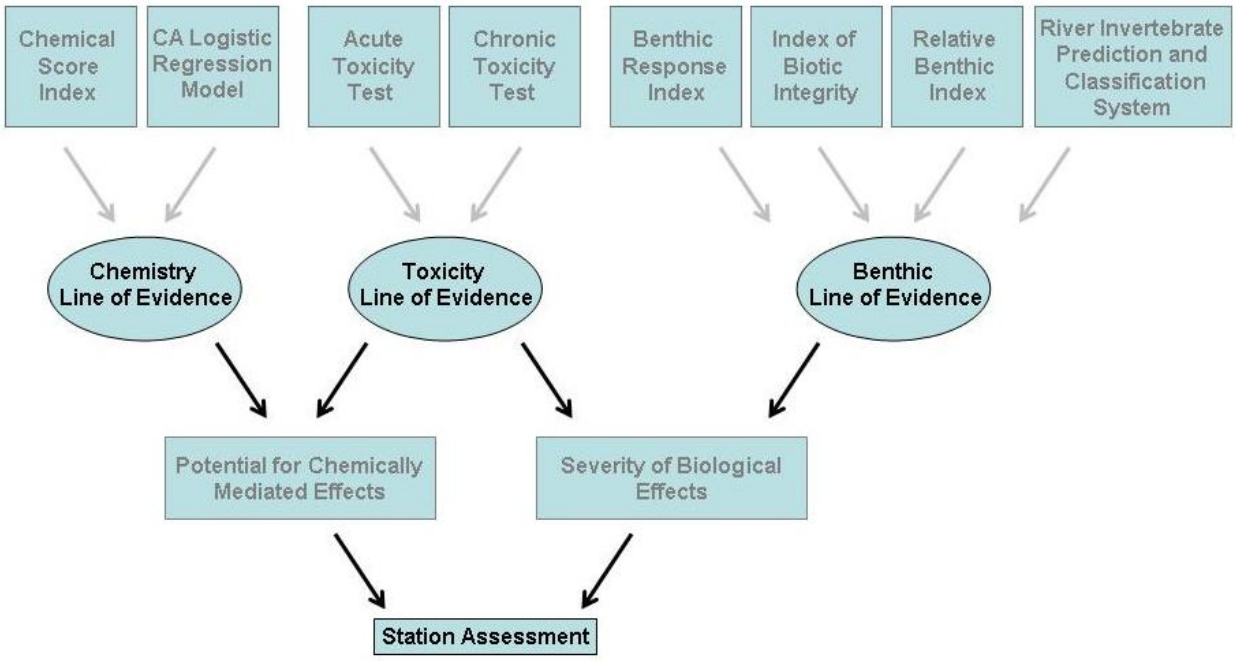


**Figure 6.1. Stages of sediment-quality assessment within the CASQO framework.**

## LOE Integration Method

Chapters 3 through 5 of this manual described the methods for determining the Chemistry, Toxicity, and Benthic lines of evidence (LOEs), each of which are based upon multiple indices or tests (Figure 6.1). Each LOE is represented by one of four possible categories (*e.g.*, Nontoxic, Low, Moderate, or High for the Toxicity LOE). Consequently there are 64 possible combinations of the three LOEs. An integration framework was developed to relate each of the possible combinations to a set of six final station assessment categories:

- Unimpacted
- Likely Unimpacted
- Possibly Impacted
- Likely Impacted
- Clearly Impacted
- Inconclusive

The framework is based on a conceptual approach that consists of two key integration steps. First, the results for each LOE are used to classify the sediment with respect to two key elements of ecological assessment: 1) Is there biological degradation at the site, and 2) Is chemical exposure at the site high enough to potentially result in a biological response? Second, the

biological effects and chemical exposure classification results are combined to arrive at the final station assessment.

Details of the conceptual approach and its validation are described in the sediment quality objective (SQO) policy document (SWRCB 2007) and Bay and Weisberg 2008. The efficacy of the framework was assessed by applying it to data from 25 sites and comparing the site classifications to those of six experts who provided the same data. The framework produced an answer that better matched the median classification of the experts than did five of the six experts. Moreover, the bias in response was less than that obtained from some of the experts, and the errors were relatively evenly divided between sites classified as more impacted or less impacted than the median expert classification. The framework was also applied and found to effectively distinguish sites from known degraded and reference areas within California.

Determination of the final station assessment category is a simple process once the result for each LOE has been determined. The category results for the LOEs are matched to a table of all possible combinations (Table 6.1) and the corresponding final station assessment is selected. To use Table 6.1, first compile the Chemistry LOE, Toxicity LOE, and Benthic LOE results for the station sampled. Starting with the Chemistry LOE, locate the section of the table that corresponds to whether the station's sediment chemistry exposure category was Minimal, Low, Moderate, or High. Then, within the appropriate Chemistry LOE category of the table, identify the region corresponding to the Benthic LOE category for that site (*i.e.*, Reference, Low, Moderate, or High). Finally, within the appropriate Chemistry LOE and Benthic LOE category combination, identify the Toxicity LOE category for that site (*i.e.*, Nontoxic, Low, Moderate, or High). The row with the appropriate combination of MLOEs for a given station yields that station's sediment condition category (the final column). Additional instructions and software tools to conduct all of the required analyses and comparisons are available on the Sediment Quality Assessment Tools page of the SCCWRP web site (http://www.sccwrp.org/view.php?id=565).

**Table 6.1. Station assessment categories resulting from each possible MLOE combination.**

| Line of Evidence Category Combination | Chemistry LOE: Sediment Chemistry Exposure | Benthic LOE: Benthic Community Condition | Toxicity LOE: Sediment Toxicity | Station Assessment (Site Condition) |
|---|---|---|---|---|
| 1 | Minimal | Reference | Nontoxic | Unimpacted |
| 2 | Minimal | Reference | Low | Unimpacted |
| 3 | Minimal | Reference | Moderate | Unimpacted |
| 4 | Minimal | Reference | High | Inconclusive |
| 5 | Minimal | Low | Nontoxic | Unimpacted |
| 6 | Minimal | Low | Low | Likely unimpacted |
| 7 | Minimal | Low | Moderate | Likely unimpacted |
| 8 | Minimal | Low | High | Possibly impacted |
| 9 | Minimal | Moderate | Nontoxic | Likely unimpacted |
| 10 | Minimal | Moderate | Low | Likely unimpacted |
| 11 | Minimal | Moderate | Moderate | Possibly impacted |
| 12 | Minimal | Moderate | High | Likely impacted |
| 13 | Minimal | High | Nontoxic | Likely unimpacted |
| 14 | Minimal | High | Low | Inconclusive |
| 15 | Minimal | High | Moderate | Possibly impacted |
| 16 | Minimal | High | High | Likely impacted |
| 17 | Low | Reference | Nontoxic | Unimpacted |
| 18 | Low | Reference | Low | Unimpacted |
| 19 | Low | Reference | Moderate | Likely unimpacted |
| 20 | Low | Reference | High | Possibly impacted |
| 21 | Low | Low | Nontoxic | Unimpacted |
| 22 | Low | Low | Low | Likely unimpacted |
| 23 | Low | Low | Moderate | Possibly impacted |
| 24 | Low | Low | High | Possibly impacted |
| 25 | Low | Moderate | Nontoxic | Likely unimpacted |
| 26 | Low | Moderate | Low | Possibly impacted |
| 27 | Low | Moderate | Moderate | Likely impacted |
| 28 | Low | Moderate | High | Likely impacted |
| 29 | Low | High | Nontoxic | Likely unimpacted |
| 30 | Low | High | Low | Possibly impacted |
| 31 | Low | High | Moderate | Likely impacted |
| 32 | Low | High | High | Likely impacted |
| 33 | Moderate | Reference | Nontoxic | Unimpacted |
| 34 | Moderate | Reference | Low | Likely unimpacted |
| 35 | Moderate | Reference | Moderate | Likely unimpacted |
| 36 | Moderate | Reference | High | Possibly impacted |
| 37 | Moderate | Low | Nontoxic | Unimpacted |
| 38 | Moderate | Low | Low | Possibly impacted |
| 39 | Moderate | Low | Moderate | Possibly impacted |
| 40 | Moderate | Low | High | Possibly impacted |
| 41 | Moderate | Moderate | Nontoxic | Possibly impacted |
| 42 | Moderate | Moderate | Low | Likely impacted |
| 43 | Moderate | Moderate | Moderate | Likely impacted |
| 44 | Moderate | Moderate | High | Likely impacted |
| 45 | Moderate | High | Nontoxic | Possibly impacted |

**Table 6.1 Continued.**

| Line of Evidence Category Combination | Chemistry LOE: Sediment Chemistry Exposure | Benthic LOE: Benthic Community Condition | Toxicity LOE: Sediment Toxicity | Station Assessment (Site Condition) |
|---|---|---|---|---|
| 46 | Moderate | High | Low | Likely impacted |
| 47 | Moderate | High | Moderate | Likely impacted |
| 48 | Moderate | High | High | Likely impacted |
| 49 | High | Reference | Nontoxic | Likely unimpacted |
| 50 | High | Reference | Low | Likely unimpacted |
| 51 | High | Reference | Moderate | Inconclusive |
| 52 | High | Reference | High | Likely impacted |
| 53 | High | Low | Nontoxic | Likely unimpacted |
| 54 | High | Low | Low | Possibly impacted |
| 55 | High | Low | Moderate | Likely impacted |
| 56 | High | Low | High | Likely impacted |
| 57 | High | Moderate | Nontoxic | Likely impacted |
| 58 | High | Moderate | Low | Likely impacted |
| 59 | High | Moderate | Moderate | Clearly impacted |
| 60 | High | Moderate | High | Clearly impacted |
| 61 | High | High | Nontoxic | Likely impacted |
| 62 | High | High | Low | Likely impacted |
| 63 | High | High | Moderate | Clearly impacted |
| 64 | High | High | High | Clearly impacted |

## Example Station Assessment

The examples that were presented in Chapters 3, 4, and 5 had the following outcomes for the three LOEs:

- Chemistry LOE = ***Moderate***
- Benthic LOE = ***Low***
- Toxicity LOE = ***Low***

Applying this information to the matrix, we see that this combination corresponds to Line of Evidence Category Combination in row 38, which yields a Station Assessment (Site Condition) category of ***Possibly Impacted.*** The text in this row of the table is bold and italicized in Table 6.2, which is a subset of Table 6.1 shown for illustrative purposes.

**Table 6.2. Subset of rows from Table 3.2 showing the results from the sample dataset.**

| Line of Evidence Category Combination | Chemistry LOE: Sediment Chemistry Exposure | Benthic LOE: Benthic Community Condition | Toxicity LOE: Sediment Toxicity | Station Assessment (Site Condition) |
|---|---|---|---|---|
| 36 | Moderate | Reference | High | Possibly impacted |
| 37 | Moderate | Low | Nontoxic | Unimpacted |
| ***38*** | ***Moderate*** | ***Low*** | ***Low*** | ***Possibly impacted*** |
| 39 | Moderate | Low | Moderate | Possibly impacted |
| 40 | Moderate | Low | High | Possibly impacted |

## Interpretation of Station Assessment Results

There are six possible station condition categories that can result from application of the CASQO assessment approach. The interpretation of these categories, in terms of certainty and magnitude of contaminated sediment impacts to aquatic life, are provided in Table 6.3. These categories reflect the reality that multiple lines of evidence may disagree and that the degree of agreement provides important information regarding the certainty of the assessment and magnitude of effects.

**Table 6.3. CASQO Sediment Condition categories and interpretation.**

| Condition Category | Interpretation |
|---|---|
| Unimpacted | Confident that contamination is not causing significantly adverse impacts to aquatic life in the sediment |
| Likely Unimpacted | Contamination is not expected to cause adverse impacts to aquatic life in the sediment, but some disagreement among lines of evidence reduces certainty that the site is unimpacted |
| Possibly Impacted | Contamination at the site may be causing adverse impacts to aquatic life in the sediment, but the level of impact is either small or is uncertain because of disagreement among lines of evidence |
| Likely Impacted | Evidence of contaminant-related impacts to aquatic life in the sediment is persuasive, in spite of some disagreement among lines of evidence |
| Clearly Impacted | Sediment contamination at the site is causing clear and severe adverse impacts to aquatic life in the sediment |
| Inconclusive | Disagreement among lines of evidence suggests that either data are suspect or additional information is needed for classification |

### *Relationship to Sediment Quality Objectives*

The categories representing the lowest estimated levels of impact to aquatic life in the sediment are *Unimpacted* and *Likely Unimpacted*. Stations classified within these two categories meet the SQO for aquatic life according to the current draft of the SQO policy. The *Possibly Impacted*, *Likely Impacted*, and *Clearly Impacted* categories indicate, with increasing levels of severity and confidence, that sediment contamination impacts to aquatic life exist. Stations classified within these three categories do not meet the SQO for aquatic life according to the current draft of the SQO policy.

The *Possibly Impacted* station assessment is the least certain of all categorizations, and therefore requires the most caution during interpretation. Stations may be classified as *Possibly Impacted* due to low levels of effect for each LOE, indicating a low magnitude of impacts. Alternatively, a *Possibly Impacted* classification may be the result of a large disagreement between LOEs, potentially due to confounding factors or noncontaminant stressors. Additional monitoring or specialized investigations may be useful in confirming the level of impact at these stations before deciding on management actions.

### *Inconclusive Results*

The *Inconclusive* category is assigned when the LOE results show an extreme level of disagreement that cannot be explained by our current understanding of sediment quality assessment. An example of this situation is when a high level of toxicity is present, but there is no evidence of contaminant exposure and the benthic community shows no evidence of disturbance. When this occurs, additional information is needed to make a reliable determination. Stations classified as *Inconclusive* should not be used to evaluate attainment of the SQO or the condition of a water body.

## Use of the Assessment Results

The California Sediment Quality Objectives (CASQO) assessment framework provides a standardized and comparable description of sediment quality that can be used in a variety of applications. The SQO policy document (SWRCB 2007) describes the types of intended applications and limitations. The primary anticipated uses fall into two categories: monitoring for water body assessment and receiving water limits. Assessment monitoring is typically used for applications such as regional surveys and evaluation of water bodies for listing as 303(d) impaired regions. Receiving water limits are components of various types of discharge permits and are used for regulatory purposes. The specific applications and interpretation of the results for regulatory purposes must be determined by the appropriate regulatory agencies.

The CASQO and other types of sediment quality assessment results may also useful for other programs, such as the development of TMDLs. However, it is important to recognize that the CASQO assessment does not identify the cause of impacts to the benthic community and the chemical indices making up the Chemistry LOE are not equivalent to effects thresholds for specific contaminants. The CASQO assessment results are intended to be used as a descriptor of

sediment quality with respect to contaminant effects, but not as clean up criteria or a determination of the specific cause of water body impairment.

Additional studies are often necessary in order to identify the cause of sediment contamination impacts and determine the appropriate actions needed to improve sediment quality. The specifics of such studies can be varied and are often determined by many factors, including site specific conditions, sources of contamination, and the objectives of the program. It is beyond the scope of this manual to describe the specifics of study design for such varied applications. However, the identification of the cause of the sediment quality impact (stressor identification) is an important next step for many applications.

## Stressor Identification

The development of tools and guidance for stressor identification is underway by several organizations and no standard guidance is yet available that addresses all aspects of the process. The following are recommendations and additional information to assist in conducting stressor identification. These recommendations are for information purposes only and do not represent regulatory requirements that are part of the SQO policy.

Three types of additional information are needed to assist in the planning of actions to improve sediment quality: 1) confirmation that pollutants are indeed the basis for the impact; 2) establishment of what specific chemical(s) is the cause of impact; 3) identification of the source of the chemical(s). The USEPA has set forth guidelines for critically reviewing data on impaired sites, listing candidate causes, characterizing the causes, and evaluating the confidence level of the identification (USEPA 2000).

A variety of approaches are potentially useful for investigating the causes of impacted sediment quality in bays and estuaries with respect to aquatic life. All of these approaches may not be needed or appropriate for a particular investigation; the design of a study should be done on a site-specific basis. The approaches appropriate for a given waterbody or site will depend on several factors, including the magnitude and nature of the impact (*e.g.*, toxicity or benthic community disturbance) and the suspected contaminants of concern.

### *Confirmation of Chemical Linkage*

The MLOE assessment establishes linkage to sediment contaminants, but the lack of confounding factors (*e.g.*, physical disturbance, non-pollutant constituents) should be confirmed. Impacts caused by physical factors at a site can be of many forms. Examples of physical stressors include reduced salinity from fresh water inputs (*e.g.*, runoff or wastewater discharge), impacts from dredging, very fine or coarse grain size and prop wash from passing ships. These types of stressors may produce a non-reference condition in the benthic community that is similar in appearance to that caused by contaminants. If impacts to a site are primarily due to physical disturbance, the LOE characteristics will likely show a degraded benthic community with little or no toxicity and low chemical concentrations. Supplemental information on habitat characteristics, dredging history, sediment particle size, and commercial/recreational use of the site should be evaluated if physical stressors are suspected.

There are a few sediment constituents whose presence may cause toxicity or benthic community disturbance, but that are not considered as pollutants for the MLOE assessment. These constituents, such as total organic carbon, nutrients and pathogens, may have sources similar to chemical pollutants (*e.g.,* wastewater treatment plant effluent) and thus produce a misleading correlation between chemical concentration and effects. Chemical and microbiological analysis will be necessary to determine if these constituents are present. The LOE characteristics for this type of stressor would likely be a degraded benthic community with possibly an indication of toxicity (*e.g.,* due to ammonia or hydrogen sulfide), and low chemical concentrations. Supplemental data on sediment concentrations or inputs of organic carbon, nutrients, or other non-target constituents should be reviewed if impacts due to non-pollutant stressors are suspected.

The type of impact that the SQO program is designed to identify is that caused by a significant exposure to chemical pollutants. This type of exposure would have LOE characteristics of a degraded benthic community, presence of toxicity, and elevated chemical concentrations. Depending on the level of agreement between LOE, forensic chemistry and other types of analyses may be needed to confirm that chemical exposure is the cause of impacted sediment quality. The site's chemical history should be examined in order to identify effluent discharges, spills or other sources of chemical contamination. Tools such as geographical information systems (GIS) and other landscape information can play a key role in that examination. It may be necessary to measure alternate suites of chemicals after more is known about a site's history. There are many types of organic chemicals that are not measured in the normal suite of priority pollutants that may be a cause of toxicity (*e.g.,* organophosphorus and pyrethroid pesticides). Body burden data should be examined from animals exposed to the site's sediment to indicate if contaminants are being accumulated and to what degree.

A variety of statistical methods may be helpful in confirming a linkage between chemical exposure and biological effects. Chemical-specific mechanistic benchmarks, such as those based on equilibrium partitioning, may be used to confirm the presence of biologically significant sediment chemistry concentrations (USEPA 2003). Comparison of the sediment chemistry data to the concentrations of contaminants measured in other locations may be helpful in verifying whether there is a plausible association between specific contaminants and biological effects. An association between variations in chemical concentration and a biological effect does not indicate the cause of impacts, but such comparisons can be useful in gaining perspective on the magnitude of contamination and in prioritizing constituents for further investigation (*e.g.*, pesticide concentrations are far below levels associated with a high probability of toxicity in other locations). Data from multiple stations within the area of interest should be examined to determine if correlations are present between measurements of sediment chemistry and biological effect.

### *Identification of Cause*

Once it is confirmed that chemical contamination is the cause of a site's impairment, the specific chemicals or chemical groups responsible must be identified before management alternatives can be developed. A combination of approaches that include statistical, biological, and chemical analyses may be needed. These approaches fall into four general categories:

- Statistical analysis
- Laboratory toxicity identification evaluations
- Bioavailability analyses
- Confirmation

Statistical Analysis

Statistical methods include correlations between individual chemicals and biological endpoints (toxicity and benthic community). A significant correlation does not indicate a causal relationship, but provides additional evidence useful for prioritizing contaminants of interest. Care must be taken when interpreting correlative relationships because individual chemicals often correlate with one another, as well as with sediment physical characteristics, such as grain size. Another statistical method is gradient analysis. For this, comparisons are made between samples collected at various distances from a potential chemical source or hotspot to examine patterns in chemical concentrations and biological responses. As the concentrations of causative agents decrease, so should biological effects.

Toxicity Identification Evaluation (TIE)

A toxicological method for determining the cause of impairment is the use of toxicity identification evaluations (TIE). During a TIE, sediment samples are manipulated chemically or physically to remove classes of chemicals or render them biologically unavailable. Following the manipulations, animal exposures are performed to determine if toxicity has been removed. At the present time, there is limited detailed guidance on performing sediment TIEs. The USEPA has published guidance for some aspects of sediment TIEs (USEPA 2007). Methods for the removal of organics, metals, and ammonia from whole sediments are available from the scientific literature (Burgess *et al.* 2000, 2003; Ho *et al.* 1999, 2002, 2004; Lebo *et al.* 1999, 2000; Pelletier *et al.* 2001).

Existing sediment TIE methods are most effective at determining cause based on broad classes of chemicals, such metals or non-polar organics, rather than individual chemicals. Powdered coconut charcoal has been successfully used to sorb organic contaminants rendering them nontoxic (Ho *et al.* 2004). While this treatment is very effective at reducing or eliminating toxicity, due to the extremely fine nature of the charcoal it cannot be recovered from the sediment and analyzed chemically to determine which constituents it has bound. Carbonaceous, nonpolar resins have also been added to the sediment to bind organic chemicals (Kosian *et al.* 1999). While these resins are not always as effective at removing toxicity as the charcoal, they offer the advantage of being recoverable from the sediment for analysis to determine what chemicals were bound. Semipermeable membrane devices (SPMDs) have also be used to remove organic compounds from sediments (Lebo *et al.* 1999, Lebo *et al.* 2000). These devices consist of polyethylene tubing or lipid filled polyethylene tubing (known as detox spiders) that are added to the sediment. The SPMDs, like the resins, can be recovered for chemical analysis.

Cation exchange resin may be added to sediments to remove toxicity caused by cationic metals (Burgess *et al.* 2000). The cation exchange resins can be extracted with acids and the extracts

analyzed to determine which metals were removed from the sediments. Prior research has shown that metals are rarely identified as the source of toxicity in whole sediment (Ho *et al.* 2002). This may be due to the higher concentrations of sulfides that are commonly associated with contaminated sediments, which bind the metals and make them biologically unavailable.

There are multiple TIE procedures for the removal of ammonia from sediments. The first is biological removal in which pieces of the alga *Ulva lactuca* are added to the overlying water (Pelletier *et al.* 2001). The algae can absorb high levels of ammonia, but may also remove other contaminants. The other treatment that has been found to be equally effective is the addition of zeolite to the sediment (Burgess *et al.* 2003). This treatment has been found to also remove some cationic metals from the sample. A less effective treatment for ammonia is aeration. It has been found that aeration is not very effective at normal pH, but removes ammonia effectively when the pH is adjusted to 10 (Burgess *et al.* 2003).

Organophosphate and pyrethroid pesticides are contaminants of increasing concern in sediments. Some methods are available that are helpful for identifying toxicity caused by these classes of pesticides. Addition of the metabolic inhibitor piperonyl butoxide (PBO) to the overlying water in a sediment toxicity test chamber has been found to be effective for removing toxicity caused by organophosphorus pesticides (USEPA 1993). In the presence of pyrethroids, PBO acts as a synergist increasing toxicity over that of an untreated sample (Wheelock *et al.* 2004). If pyrethroids are suspected, an enzyme, carboxylesterase can be added, which will reduce or eliminate toxicity by rapidly degrading the pesticide. This method has only recently been used successfully with freshwater whole sediments (Phillips *et al.* 2005) and needs to be tested for marine samples.

While pore water tests are not recommended for the initial MLOE assessment of sediment quality in this program, they are a valuable tool for helping to identify the cause of toxicity (Carr and Nipper 2003). Pore water samples are amenable to all of the aqueous sample TIE methods that are available (USEPA 1996). Currently there are more tools available for the aqueous matrix than there are for whole sediment. The use of solid phase extraction columns for the removal of organics and metals is a valuable tool that is not available for use with whole sediments. These columns can be eluted to remove the extracted chemicals, fractionated, and tested using add-back toxicity tests to provide a much finer discrimination of causative agents.

Bioavailability Analyses

Chemical contaminants may be present in the sediment but not biologically available to cause toxicity or degradation of the benthic community. There are several measures of bioavailability that can be made. Chemical and toxicological measurements can be made on pore water to determine the availability of sediment contaminants. The potential bioavailability of metals can be assessed through sulfide analysis. Measurement of acid volatile sulfides and simultaneously extracted metals analysis can be conducted to determine if sufficient sulfides are present to bind divalent metals and maintain porewater concentrations below toxic levels (Berry *et al.* 1996). Similarly, nonpolar organic compounds can be tightly bound to sediment organic carbon, which limits bioavailability. Several methods are being developed and evaluated to assess the bioavailability of organic contaminants. These methods include solid phase microextraction

(Mayer *et al.* 2000), extractions using animal digestive fluids (Weston and Maruya 2002), and weak chemical extractions (Tang *et al.* 1999).

Confirmation

After specific chemicals are identified as likely causes of impairment, analyses should be conducted to verify the results. For example, body burden analysis can be conducted on animals exposed to the sediment. The concentrations in the animals' tissues may then be compared to established toxicity thresholds to determine if critical body residues are exceeded. Sediments can be spiked with the suspected chemicals to verify that they are indeed toxic at the concentrations observed in the field. Spiked sediment studies must be carefully designed to take into consideration the geochemistry of the site sediments, form of the contaminant, and equilibration of the contaminant among binding phases. Otherwise, the spiked sediment results may not be applicable to the study site. Alternatively, animals can be transplanted to study sites for *in situ* toxicity and bioaccumulation testing.

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# APPENDIX A: USING THE STATISTICAL ANALYSIS SYSTEM (SAS) FOR RIVPACS CALCULATIONS

ftp://ftp.sccwrp.org/pub/download/DOCUMENTS/TechnicalReports/582_CASQO_Draft_AppendixA.pdf

# APPENDIX B: USING THE WEB SITE AT UTAH STATE UNIVERSITY'S CENTER FOR MONITORING AND ASSESSMENT OF FRESHWATER ECOSYSTEMS FOR RIVPACS CALCULATIONS

ftp://ftp.sccwrp.org/pub/download/DOCUMENTS/TechnicalReports/582_CASQO_Draft_AppendixB.pdf

# APPENDIX C: SORTING AND RE-ANALYSIS FORMS

ftp://ftp.sccwrp.org/pub/download/DOCUMENTS/TechnicalReports/582_CASQO_Draft_AppendixC.pdf

# APPENDIX D: BENTHIC HABITAT TYPES

ftp://ftp.sccwrp.org/pub/download/DOCUMENTS/TechnicalReports/582_CASQO_Draft_AppendixD.pdf