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ENVIRONMENTAL MONITORING AND ASSESSMENT PROGRAM (EMAP)
LABORATORY METHODS MANUAL
ESTUARIES

VOLUME 1 - BIOLOGICAL AND PHYSICAL ANALYSES

edited by

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DISCLAIMER

This document is intended to document analytical methods for use by laboratories conducting analyses for the Environmental Monitoiring and Assessment Program-Estuaries. Mention of trade names, products, or services does not convey, and should not be interpreted as conveying, official EPA approval, endorsement, or recommendation.

NOTICE

This document replaces the previous EMAP-Estuaries Laboratory Methods Manual (referenced as U.S. EPA, 1993 or Klemm *et al*., 1993) which was never officially released other than in draft form. That document was split into two volumes to conserve on paper (most laboratories conducting analyses do not need ALL methods), and reformatted somewhat (for appearance only). Note that the majority of the text remains unchanged from the 1993 document. It is distributed unbound but hole-punched so individual sections can be updated and distributed as necessary.

This document is Volume I of a two-part series. The second volume of the EMAP-Estuaries Laboratory Methods Manual presents methods for the chemical analyses of sediments and tissue.

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SECTION 1

INTRODUCTION

1. BACKGROUND

1.1 The U.S. Environmental Protection Agency is developing the Environmental Monitoring and Assessment Program (EMAP) to determine the current status, extent, changes and trends in the condition of our nation's ecological resources on regional and national scales. The nation's ecological resources are a national heritage, as essential to the country now and in the future as they have been in the past. Data indicate that regional and international environmental problems may be endangering these essential resources. The potential threats include acid rain, ozone depletion, nonpoint-source pollution, and climate change.

1.2 Unfortunately, the status of the national environment is not well documented, rendering it impossible to assess quantitatively where resources may be degrading and at what rate the degradation may be occurring. The EPA Science Advisory Board (SAB) recognized this deficiency and recommended in 1988 that EPA initiate a program that would monitor ecological status and trends, as well as develop innovative methods for anticipating emerging problems before they reach crisis proportions. EPA's response to the SAB's recommendations is EMAP.

2. OBJECTIVES

2.1 The primary goal of EMAP is to provide environmental decision makers with statistically valid interpretive reports describing the health of our nation's ecosystems. Knowledge of the health of our ecosystems will give decision makers and resource managers the ability to make informed decisions, set rational priorities, and make known to the public the costs, benefits, and risks of proceeding or refraining from implementing specific environmental regulatory actions. EMAP's ecological status and trends data will allow decision makers to assess objectively whether or not the nation's ecological resources are responding positively, negatively, or not at all, to the regulatory programs put in place ostensibly to benefit them.

2.2 To accomplish its goals, EMAP is to document the condition of the nation's forest, wetlands, estuarine and coastal waters, inland surface waters, Great Lakes, agricultural lands, and arid lands in an integrated manner, on a continuing basis. Although EMAP is designed and funded by EPA's Office of Research and Development (ORD), other offices and regions within EPA and other federal agencies have contributed to its development and will participate in the collection and use of EMAP data. When fully implemented, EMAP will form a complex national monitoring network, with a large proportion of the data collection and analysis being accomplished by other federal, state, and local agencies.

2.3 The following four objectives guide EMAP (from Thornton *et al.*, 1993):

- Estimate the current status, trends, and changes in selected indicators of the Nation's ecological resources on a regional basis with known confidence.
- Estimate the geographic coverage and extent of the Nation's ecological resources with known confidence.
- Seek associations between selected indicators of natural and anthropogenic stresses and indicators of ecological resources.
- Provide annual statistical summaries and periodic assessments of the Nation's ecological resources.

3. SCOPE

3.1 EMAP-Estuaries (EMAP-E) is that part of the overall program which is monitoring the health of estuaries and marine waters along our nation's coastline. Estuaries, which occur in tidally-influenced portions of rivers and embayments, represent a vital component of near-coastal ecosystems. Estuaries form a key ecological link between the nation's rivers and the coastal waters of the continental shelf. Estuarine environments encompass tidal wetlands; submerged aquatic vegetation communities; and inlets, bays, and lagoons. Estuaries provide critical spawning and nursery habitat for commercial fish and shellfish, while the land around these ecosystems is becoming highly populated. These valuable and threatened ecosystems are easily abused. Estuaries directly receive much of the wastewater, after it has been treated, that is generated by homes, businesses, and industries in estuarine watersheds. In addition, effluent or runoff that enters rivers at points far from the coastline eventually can reach estuarine environments.

3.2 EMAP-E has divided all the nation's coastline into discrete regions for study. The first of these regions to be studied, called the Virginian Province, extends from Cape Cod, Massachusetts to Cape Henry, Virginia. Study began in Virginian Province bays and estuaries in the summer of 1990. Monitoring in the Gulf of Mexico (Louisianian Province) began in the summer of 1991. Sampling of other regions will be phased in over the next several years, until all of the coastal waters in the country are sampled yearly.

4. APPROACH

4.1 Assessing the status and trends for the nation's estuarine and coastal ecological resources requires data collected in a standardized manner, over large geographic scales, for long periods of time. Such assessments cannot be accomplished solely by aggregating data from the many individual, short-term monitoring programs that have been conducted in the past and are being conducted currently. Differences in the parameters measured, the collection methods used, timing of sample collection, and program objectives severely limit the usefulness of historical monitoring data for conducting regional and national status and trends assessments. The EMAP-E program

will monitor a defined set of parameters *(i.e*., indicators of estuarine and coastal environmental quality) on a regional scale, over a period of decades, using standardized field sampling and laboratory methods with a probability-based sampling design. These characteristics distinguish EMAP-E from other monitoring programs and will provide the data for preparing the regional and national scale assessments that are needed to address the environmental issues of the 1990's and beyond.

4.2 EMAP-E does not have the resources to monitor all ecological parameters of concern to the public, Congress, scientists, and decision makers. Therefore a defined set of parameters that serve as indicators of environmental quality are being measured. Categories of indicators identified and sampled are as follows:

- Biotic condition indicators - Measurements that quantify the integrated response of ecological resources to individual or multiple stressors. Included are benthic species composition, abundance and biomass; gross pathology of fish; fish species composition and abundance; relative abundance of large burrowing bivalves; and histopathology of fish.

- Abiotic condition indicators - Physical, chemical, and biological measurements that quantify pollutant exposure, habitat degradation, or other causes of degraded ecological condition. Included are sediment contaminant concentration; sediment toxicity; contaminants in fish flesh; contaminants in large bivalves; and continuous and point measurements of dissolved oxygen concentration.

- Habitat indicators - Physical, chemical, and biological measurements that provide basic information about the natural environmental setting. Included are sediment characteristics; water salinity, temperature, pH, depth, and clarity; chlorophyll *a* fluorescence and the amount of photosynthetically active radiation (PAR) in the water column.

4.3 Recommended protocols for those indicator parameters that are measured in the laboratory are presented in the later sections of this document and in Volume II - Chemistry Methods. Protocols for indicator parameters collected or measured in the field are contained in EMAP-E Field Operations Manuals (Macauley, 1994; Reifsteck *et al*., 1993).

5. REFERENCES

Macauley, J. 1994. Environmental Monitoring and Assessment Program - Estuaries: 1994 Louisianian Province Field Operations and Safety Manual. Environmental Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Gulf Breeze, FL.

Reifsteck, D.R., C.J. Strobel and D.K. Keith. 1993. Environmental Monitoring and Assessment Program - Estuaries: 1993 Virginian Province Field Operations and Safety Manual. U.S. Environmental Protection Agency, Office of Research and Development, Environmental Research Laboratory, Narragansett, RI. June 1993.

Thornton, K.W., D.E. Hyatt, and C.B. Chapman, eds. 1993. Environmental Monitoring and Assessment Program Guide. EPA/620/R-93/012, Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Assessment Program, EMAP Research and Assessment Center.

SECTION 2

SEDIMENT TOXICITY TEST METHODS

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Subsections

1. INTRODUCTION

1.1 In the EMAP-Estuaries program, the acute toxicity of surface sediments will be assessed as a measure of the biological effects of sediment contaminants. In general, sediment toxicity tests provide information that is independent of chemical characterizations and ecological surveys (Chapman, 1988). These tests have proven to be effective in both marine and freshwater environments (Chapman, 1988; Swartz, 1987) and have become an integral part of many benthic assessment programs (Swartz, 1989). They have been used for permitting programs, site ranking for remediation, recovery studies following management actions, and trends monitoring. A particularly important application is in programs seeking to establish contaminant-specific effects.

1.2 The marine and estuarine amphipod sediment toxicity tests performed on EMAP-Estuaries will follow standard ASTM guidelines (ASTM, 1991) and EPA methods (U.S. EPA, 1994). For a typical bioassay, 200 mL of surface sediment (top 2 cm) from homogenized grab samples collected at each sampling site will be placed in one-liter canning jars covered with 600 ml of water. The bioassays will be conducted for 10 days, under static conditions, at a constant temperature of 20°C and a salinity of 30 ± 2‰. Five replicate test chambers will be used for the sediment from each station.

1.3 The east coast marine amphipod *Ampelisca abdita* will be employed as the primary test species in the sediment toxicity tests. This species has been shown to be both acutely (Breteler *et al*., 1989; DiToro *et al*., 1990; Rogerson *et al*., 1985) and chronically (Scott and Redmond, 1989) sensitive to contaminated sediments. In addition, it has been shown to be comparable in sensitivity to more commonly-used test species like *Rhepoxynius abronius* and *R*. *hudsoni* (DiToro *et al*., 1990), and has been successfully tested using contaminated low salinity sediments. Because *Ampelisca* is a tube dweller, it is tolerant of a wider range of sediment types than *Rhepoxynius* (Long and Buchman, 1989).

1.4 Because the sediment toxicity indicator needed by EMAP-Estuaries must be comparable across all salinities, all tests will be conducted using *Ampelisca abdita* at a salinity of 30 ± 2‰.

1.5 In the Louisianian Province (the U.S. Coastal Gulf of Mexico), mysids (*Mysidopsis bahia*) will also be used as a primary test species, in addition to *A*. *abdita*, for sediment toxicity tests. Mysids are small shrimp-like crustaceans with demonstrated sensitivity to environmental contaminants. The U. S. EPA Environmental Research Laboratory at Gulf Breeze, FL (ERL-GB) has developed culturing techniques and toxicity testing methods (acute and life-cycle) for *Mysidopsis bahia* (U.S. EPA, 1978; U.S. EPA, 1987). Test procedures, based on USEPA-approved guidelines for conducting solid-phase bioassays (USEPA/CE, 1977), have been adapted for EMAP-Estuaries and will be 96-hr static tests with aeration.

1.6 Also, during the first year of monitoring in the Louisianian Province, commercially important, penaeid shrimp (*Penaeus duorarum* or *P*. *aztecus*) will be tested with sediment collected from 16 Indicator Testing and Evaluation (ITE) sites; cost and logistical constraints prohibit testing penaeid shrimp on a Province-wide scale. Sediment tests with post-larvae or juvenile penaeid shrimp will be conducted by using

the same methods as described for mysids. Test results will be compared and evaluated for inferences linking the observed toxicity to mysids to that for penaeids.

1.7 The sediment toxicity tests also will include clean control sediments with grain size and other natural features matching those of the test sediment, as well as "water only" 96-hr exposure tests using the reference toxicant sodium dodecyl sulfate (SDS) at various concentrations to determine LC50 values on a continual basis (to assess both interlaboratory precision and the relative sensitivity of a given batch of test organisms).

2. SCOPE AND APPLICATION

2.1 A number of sediment bioassay guides (U.S. EPA/CE, 1977;U.S. EPA, 1994; ASTM, 1991; Swartz *et al*., 1979; Swartz *et al*., 1985) describe procedures for obtaining laboratory data concerning the short-term adverse effects of potentially contaminated sediment, or of a test material experimentally added to contaminated or uncontaminated sediment, on marine or estuarine infaunal amphipods during static 10-day exposures. These procedures are useful for testing the effects of various geochemical characteristics of sediments on marine and estuarine amphipods, and could be used to assess sediment toxicity to other infaunal taxa, although modifications of the procedures appropriate to the test species might be necessary. Procedures for 10-day static sediment toxicity tests are described herein for *Ampelisca abdita* (marine and estuarine).

2.2 This procedure is applicable to sediments containing most chemicals, either individually or in formulations, commercial products, and known or unknown mixtures. With appropriate modifications this procedure can be used to conduct sediment toxicity tests on factors such as temperature, salinity, and dissolved oxygen, and natural sediment characteristics (*e.g*., particle size distribution, organic carbon content, total solids). This method can also be used to conduct bioconcentration tests and *in situ* tests, and to assess the toxicity of potentially contaminated field sediments, or of such materials as sewage sludge, oils, particulate matter, and solutions of toxicants added to sediments. An LC50 or EC50 of toxicants or of highly contaminated sediment mixed into uncontaminated sediment can be determined. Materials either adhering to sediment particles or dissolved in interstitial water can be tested.

2.3 This test procedure is not intended to exactly simulate the exposure of benthic amphipods to contaminants under "natural" conditions, but rather to provide a convenient, rapid, standard toxicity test procedure yielding a reasonably sensitive indication of the toxicity of materials in marine and estuarine sediments.

2.4 Amphipods are an abundant component of the soft bottom marine and estuarine benthic community. They are a principal prey of many fish, birds and larger invertebrate species. Some species are predators of smaller benthic invertebrates. Others ingest sediment particles and thus are directly exposed to contaminants. Amphipods are among the first taxa to disappear from benthic communities impacted by pollution, and have been shown to be more sensitive to contaminated sediments than several other major taxa (Sax, 1984). The ecological importance of amphipods, their wide geographical distribution, ease of handling in the laboratory, and their sensitivity to contaminated sediments make them appropriate species for sediment toxicity testing.

3. SUMMARY OF METHOD

3.1 The relative toxicity of marine or estuarine sediments can be determined through a 10-day static test with solid phase sediment and overlying water in aerated 1-L glass test chambers. Mortality and sublethal effects, such as emergence from sediment, are determined after exposure of a specific number (usually 20) of amphipods to a quantity of test sediment. Response of the amphipods to the test sediment is compared with response in control sediment. A negative control or reference sediment is used to provide (a) a measure of the acceptability of the test by providing evidence of the health and relative quality of the test organisms, and the suitability of the overlying water, test conditions and handling procedures, and (b) the basis for interpreting data obtained from the test sediments.

3.2 The toxicity of field-collected sediment is indicated by the percent mortality of amphipods exposed to that sediment compared to those exposed to control sediment. The toxicity of field sediments may also be assessed by testing dilutions of a highly toxic test sediment with clean sediment to obtain information on the toxicity of proportions of that sediment.

4. DEFINITIONS

4.1 The term "sediment" is used here to denote a naturally occurring particulate material which has been transported and deposited at the bottom of a body of water. The procedures described can also be applied using an experimentally prepared substrate within which the amphipods can burrow.

4.1.1 "Clean" sediment denotes sediment which does not contain concentrations of toxicants which cause apparent stress to the test organisms or reduce their survival.

4.1.2 "Solid-phase" sediment is distinguished from elutriates and resuspended sediments in that the whole, intact sediment is used to expose the organisms, not a form or derivative of the sediment.

4.2 "Toxicity" is the property of a material or combination of materials, to adversely affect organisms (see ASTM 943).

4.3 "Exposure" is contact with a chemical or physical agent (see ASTM 943).

4.4 "Interstitial water" is the water within a wet sediment that surrounds the sediment particles. The amount of interstitial water in sediment is expressed as the percent ratio of the weight of the water in the sediment to that of the wet sediment.

4.5 "Overlying water" is the water which is added to the test chamber over the solid phase of the sediment in a toxicity test.

4.6 "Partial life-cycle test" is a test utilizing a sensitive life-stage(s) of an organism used to assess the toxicity of contaminants, and is distinguished from a complete life-cycle test which is conducted from egg to egg or beyond, or for several life-cycles (Rand and Petrocelli, 1985).

4.7 The LC50 is the statistically or graphically derived best estimate of the concentration of test material added to or contained in sediment that is expected to be lethal to 50% of the test organisms under specified conditions within the test period (see ASTM Standard E 943).

4.8 The EC50 is the statistically or graphically estimated concentration of test material in sediment that is expected to cause a measured sublethal effect (for example the inability of amphipods to rebury in clean sediment at the end of the test period), in 50% of the test organisms under specified conditions (see ASTM Standard E 943).

5. INTERFERENCES

5.1 Due to the limited time sediment toxicity tests have been practiced, the methodology continues to develop and evolve with time and research needs. Because of the developmental nature of sediment toxicity testing, there are limitations to the method described in this guide.

5.2 Results of acute sediment toxicity tests will depend, in part, on the temperature, water quality, physical and chemical properties of the test sediment, condition of the test organisms, exposure technique, and other factors. Factors potentially affecting results from static sediment toxicity tests might include items 5.2.1 to 5.2.11.

5.2.1 Alteration of field sediments in preparation for laboratory testing.

5.2.1.1 Maintaining the integrity of the sediment environment during its removal, transport and testing in the laboratory is extremely difficult. The sediment environment is composed of a myriad of micro-environments, redox gradients and other interacting physiochemical and biological processes. Many of these characteristics influence sediment toxicity and bioavailability to benthic and planktonic organisms, microbial degradation, and chemical sorption. Any disruption of this environment complicates interpretations of treatment effects, causative factors, and *in situ* comparisons.

5.2.1.2 Testing of sediments at temperatures or salinities other than those at which they were collected might affect contaminant solubility, partitioning coefficients, and other physical and chemical characteristics.

5.2.2 Interactions between the sediment particles, overlying water, interstitial water, and humic substances, and the sediment to overlying water ratio.

5.2.3 Interactions among chemicals which may be present in test sediment.

5.2.4 Photolysis and other processes degrading test chemicals.

5.2.5 Maintaining acceptable quality of overlying water.

5.2.6 Excess food may change sediment partitioning and water quality parameters.

5.2.7 Resuspension of sediment during the toxicity test.

5.2.8 Limited opportunity for biological observations during the test because organisms bury in test sediment.

5.2.9 Natural geochemical properties of test sediment collected from the field which may not be within the tolerance limits of the test organisms.

5.2.10 Recovery of test organisms from the test system.

5.2.11 Endemic organisms which may be present in field collected sediments including (a) predators, (b) species which may be the same as or closely related to the test species, and (c) microorganisms (*e.g.*, bacteria or molds) or algae colonizing sediment and test chamber surfaces.

5.3 Static tests might not be applicable to materials that are highly volatile or are rapidly biologically or chemically transformed. Furthermore, the overlying water quality may change considerably from the initial overlying water. Because the experimental chambers are aerated, the procedures can usually be applied to materials that have a high oxygen demand. Materials dissolved in interstitial waters might be removed from solution in substantial quantities by adsorption to sediment particles and to the test chamber during the test. The dynamics of contaminant partitioning between solid and dissolved phases at the initiation of the test should therefore be considered, especially in relation to assumptions of chemical equilibrium.

6. SAFETY

6.1 General Precautions

6.1.1 Field sediments to be tested, especially those from effluent areas, might contain organisms that can be pathogenic to humans. Special precautions when dealing with these sediments might include immunization prior to sampling and use of bactericidal soaps after working with the sediments.

6.1.2 Sediments collected from the field might be contaminated with unknown concentrations of many potentially toxic materials. Any potentially contaminated sediments should be handled in a manner to minimize exposure of researchers to toxic compounds.

6.2 Safety Equipment

6.2.1 Personal Safety Gear

6.2.1.1 Many materials can affect humans adversely if precautions are inadequate. Therefore, skin contact with all toxicants, overlying water, and sediments should be minimized by such means as wearing appropriate protective gloves (especially when washing equipment or putting hands into test sediments or solutions), laboratory coats, aprons, and glasses. Special precautions, such as covering test chambers and ventilating the area surrounding the chambers, should be taken when conducting tests on volatile

materials. Information on toxicity to humans (International Technical Information Institute, 1977; Sax, 1984; Patty, 1963; Hamilton and Hardy, 1974; Goselin *et al*., 1976), recommended handling procedures (Green and Turk, 1978; National Research Council, 1981; Walters, 1980; Fawcett and Wood, 1982), and chemical and physical properties of the test material should be studied before a test is begun. Special precautions might be necessary with radiolabeled test materials (National Council on Radiation Protection and Measurement, 1971; Shapiro, 1981) and with materials that are, or are suspected of being, carcinogenic (National Institutes of Health, 1981).

6.3 General Laboratory Operations

6.3.1 Mixing of toxic sediments in open containers, and loading of toxic sediments into test chambers should be done in a well-ventilated area, preferably a chemical fume hood. Face shields or protective goggles should be worn during any operations that might involve accidental splashing of sediments, such as sieving, mixing and loading into test chambers.

6.3.2 Health and safety precautions and applicable regulations for disposal of stock solutions, overlying water from test chambers, test organisms, and sediments should be considered before beginning a test (see ASTM Standard D 4447). Consideration of cost as well as detailed regulatory requirements might be necessary.

6.3.3 Cleaning of equipment with a volatile solvent such as acetone, should be performed only in a well-ventilated area in which no smoking is allowed and no open flame, such as a pilot light, is present. Cleaning equipment with acids should be done only in a well-ventilated area, and protective gloves and safety goggles should be worn.

6.3.4 To prepare dilute acid solutions, concentrated acid should be added to water, not vice versa. Opening a bottle of concentrated acid and adding concentrated acid to water should be performed only in a well-ventilated area or a chemical fume hood.

6.3.5 Use of ground fault systems and leak detectors is strongly recommended to help prevent electrical shocks because salt water is a good conductor of electricity.

7. APPARATUS AND EQUIPMENT

7.1 General Requirements

7.1.1 Test chambers containing sediment should be held in a well-lighted (at least 100 lux at the test sediment surface), constant temperature room, incubator or recirculating water bath to maintain the experimental temperature. Air used for aeration should be free of fumes, oil and water; filters to remove oil and water are desirable. The area containing the test chambers must be well ventilated and free of fumes, both to prevent contamination of test materials and to protect researchers from exposure to toxic volatile materials which might be released from the test sediments. Enclosures may be needed to ventilate the area surrounding test chambers.

7.1.2 The exposure room should be equipped with a timing device for photoperiod control. If a photoperiod other than continuous light is used, it might be desirable to incorporate a 15 to 30 minute transition period when lights go on or off to reduce stress to the organisms from sudden large changes in light intensity (Robinson *et al*., 1988; U.S. EPA, 1979a). It is also desirable to have the room temperature and light controls and the aeration on emergency power to protect the experiment in case of a power failure.

7.2 Construction Materials

7.2.1 Equipment and facilities that contact test solutions or any water or sediment into which test organisms will be placed should not contain substances that can be leached or dissolved by aqueous solutions in amounts that adversely affect test organisms. In addition, equipment and facilities that contact stock or test solutions or sediment should be chosen to minimize sorption of test materials from water.

7.2.2 Glass, Type 316 stainless steel, nylon, high density polyethylene, polycarbonate and fluorocarbon plastics should be used whenever possible to minimize dissolution, leaching, and sorption, except that stainless steel should not be used in tests on metals in salt water.

7.2.3 Concrete and rigid plastics may be used for holding tanks and in the water-supply system, but they should be soaked, preferably in flowing test water (salt or fresh), for a week or more before use (Carmignani and Bennett, 1976). Brass, copper, lead, cast iron pipe, galvanized metal, and natural rubber should not contact test seawater, stock solutions, or test sediment before or during the test.

7.2.4 Tubing used in making up test seawater or reconstituted freshwater and in aerating the test chambers should be nontoxic (Tygon® R-3603 or equivalent). New tubing should be soaked at least one week prior to use. Separate sieves, dishes, containers, and other equipment should be used to handle test sediment or other toxic materials and these should be kept and stored separately from those used to handle live animals prior to testing.

7.3 Test Chambers

7.3.1 Species-specific information on test chambers is given in Appendices C and D. The test chambers should be placed in a water bath to minimize temperature fluctuations, and should be aerated. Aeration can be provided as in Subsection 14 and 15.

7.4 Cleaning

7.4.1 Test chambers and other glassware, and equipment used to store and prepare test sea water, stock solutions, and test sediment should be cleaned before use.

7.4.2 All glassware should be cleaned before each use by washing with laboratory detergent, followed by three distilled water rinses, 10% nitric v/v (HNO_3) or hydrochloric (HCl) acid rinse, and at least two distilled water rinses.

7.5 Acceptability of Test Facilities and Equipment

7.5.1 Laboratory and bioassay temperature control equipment must be adequate to maintain recommended test temperatures. Recommended materials must be used in the fabrication of the test equipment which comes in contact with the sediment being tested. The acceptability of new holding or testing facilities should be demonstrated by conducting "non-toxicant" tests in which test chambers contain control sediment and appropriate overlying water. These tests will demonstrate whether facilities, water, control sediment, and handling techniques are adequate to result in acceptable control level survival.

7.6 Species Specific Equipment Requirements

7.6.1 See Appendix B for *Ampelisca abdita*.

8. TEST ORGANISMS

8.1 Species

8.1.1 The organism used in the test described in this manual is the marine amphipod *Ampelisca abdita*. All individuals used in the tests should be disease-free and should be positively identified to species.

8.1.2 The species of infaunal amphipod to be used in the sediment toxicity test should be selected based on availability, sensitivity to test materials, tolerance to ecological conditions (*e.g.*, temperature, salinity, and grain size), ecological importance, and ease of handling in the laboratory. The source and type of sediment being tested or the type of test to be implemented might dictate selection of a particular species.

8.1.3 Ideally, species or genera with wide geographical distributions should be selected, so that test results can be compared among laboratories with similar species. Species used should be identified with an appropriate taxonomic key, and identifications should be verified by a taxonomic authority.

8.1.4 The appendices to this document give guidance as to requirements and methods of handling for various species of amphipods. Use of the species listed in the appendices is encouraged to increase comparability of results.

8.1.5 The environmental requirements and sensitivity of a prospective test species of amphipod to test materials and to various sediment characteristics should be established before it is widely used in toxicity tests.

8.1.6 The tolerance of a test species to variations in sediment characteristics such as particle size distribution, organic enrichment, and interstitial water salinity should be established before responses can be ascribed to contaminant effects.

8.1.7 Choice of the scale of the test chamber, density of test organisms, temperature, salinity, and control sediment may have to be modified to accommodate the requirements of the test species. Required modifications should be based on conditions at the natural habitat of the species.

8.1.8 If tube-building amphipods are used in sediment toxicity testing, it should be kept in mind that the amphipods might not be directly in contact with test sediment after their tubes are built, and they might pump overlying water through their tubes rather than utilizing interstitial water. They might feed on particulate materials that either are suspended in the water column or have settled on the sediment surface, while burrowing species might feed on particles or meiofauna found within the sediment. Thus tube builders and burrowing species might have different routes of exposure to adsorbed or dissolved sediment contaminants.

8.1.9 Amphipods that emerge from the sediment and either swim in overlying water or crawl on the sediment surface might not be continually exposed to the test sediment.

8.2 Age

8.2.1 All organisms should be as uniform as possible in age and size. The age or size class for a particular species should be chosen so that sensitivity to test materials is not affected by such factors as state of maturity, reproduction, or seasonality. For EMAP-Estuaries sediment toxicity testing, juvenile *Ampelisca abdita* in the size range 3 to 5 mm should be used for testing (see appendices).

8.3 Source

8.3.1 All individuals in a test should be from the same source, because different populations of the same species might have different acute sensitivities to contaminants. Marine amphipods are usually obtained directly from a wild population in a clean area, although attempts have been made to culture some species. Collecting permits for field-collected amphipods might be required by some local and state agencies.

8.3.2 If test organisms are cultured or held for an extended period of time in the laboratory, the response of laboratory-held organisms to test materials should be compared to that of animals recently collected from the field to assure that laboratory stresses do not affect their sensitivity to test materials (Adams *et al.*, 1985). Generally the reference toxicant assay performed with each test satisfies this requirement.

8.4 Quality

8.4.1 All amphipods used in a test must be of acceptable quality. A qualified amphipod taxonomist must be consulted to ensure that the animals in the test population are all of the same species.

8.4.2 If organisms are collected from the field prior to testing, they should be obtained from an area known to be free of toxicants and should be held in clean, uncontaminated water and facilities. Organisms held prior to testing should be checked daily, and individuals which appear unhealthy or dead should be

discarded. If greater than 5% of the organisms in holding containers are dead or appear unhealthy during the 48 hours preceding a test, the entire group should be discarded and not used in the test.

8.5 Reference Toxicants

8.5.1 Whenever test organisms are obtained from an outside source (*e.g.*, field collected or obtained from an outside culture facility), their sensitivity must be evaluated with a reference toxicant in an appropriate short-term toxicity test performed concurrently with the toxicity tests of actual sediment samples. Short-term toxicity tests without sediment may be used to generate LC50 values for this purpose. If the laboratory maintains breeding cultures of test organisms, the sensitivity of the offspring should be determined in a toxicity test performed with a reference toxicant at least once a month. If preferred, this test also may be performed concurrently with the toxicity tests of sediment samples.

8.5.2 The reference toxicant sodium dodecyl sulfate (SDS) must be used for EMAP-Estuaries sediment toxicity testing using *Ampelisca abdita*.

8.6 Specific Species Requirements

8.6.1 See Appendix B Specific Requirements for *Ampelisca abdita*.

9. DILUTION WATER

9.1.1 See Appendix A for *Ampelisca abdita*.

10. REAGENTS AND CONSUMABLE MATERIALS

Reference toxicant (*e.g.*, SDS)

Small-bore pipettes

5% buffered formalin

Rose Bengal stain

11. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

11.1 See appropriate EMAP-Estuaries Field Operations Manual.

11.2 Techniques for sample collection, handling, and storage are described in the EMAP-Estuaries Field Operations Manuals (Strobel and Schimmel, 1991; Macauley, 1991). Sediment samples for toxicity testing should be chilled to 4°C when collected, shipped on ice, and stored in the dark in a refrigerator (4°C) for no longer than 30 days before the initiation of the test.

11.3 Sample containers should be made of inert materials to prevent contamination, which might result in artifactual changes in toxicity (Subsection 7, Apparatus and Equipment).

11.4 All sampling devices and any other instruments in contact with the sediments should be chemically cleaned between stations. Sediment contact with metals (including stainless steel) and plastics (including polypropylene and low density polyethylene) should be avoided as contaminant interactions (*e.g*., surface adsorption) may occur. Only sediments not in contact with the sides of the sampling device should be subsampled, composited, and subsequently homogenized using instruments composed of non-reactive (*i.e*., inert) materials. The adequacy of the field homogenization technique for sediments will be documented in a special study prior to the start of field work.

12. CALIBRATION AND STANDARDIZATION

12.1 Instruments used for routine measurements must be calibrated and standardized according to instrument manufacturer's procedures, see EPA methods: 150.1, 360.1, 170.1, and 120.1 (U.S. EPA, 1979a).

12.2 All routine chemical and physical analyses must include established quality assurance practices as outlined in Agency methods manuals (U.S. EPA, 1979a,b).

13. QUALITY ASSURANCE

13.1 Specific QA/QC guidelines for the Sediment Toxicity Test Methods are found in the most recent versions of the QA Project Plans developed for each active EMAP-Estuaries Province (Valente and Strobel, 1992; Heitmuller and Valente, 1992).

13.2 Required Controls

13.2.1 Every test requires a control treatment consisting of sediment from the amphipod collection site or other sediment known to be non-toxic to, and within the geochemical requirements of the test species. The same water, conditions, procedures, and organisms are used as in the other test treatments, except that none of the test material is added to the control sediment or water. At least five laboratory replicates of the control sediment should be included in all tests regardless of whether test sediments are replicated. This allows comparisons among experiments and among laboratories of the validity of procedures used in individual tests.

13.2.2 In addition to the standard control, if a field sediment has properties such as grain size or organic content which might exceed the tolerance range of the test species, it is desirable to include non-toxic reference sediment controls for these characteristics. The design of field surveys should include an additional field control involving five replicate samples from an area which is free from sediment contamination. This provides a site-specific basis for comparison of potentially toxic and non-toxic conditions, and can account for mortality associated exclusively with subjecting the organisms to non-native

sediments. The concentrations of chemical contaminants should be measured in these field control sediments in order to justify the assumption that they are contaminant-free.

13.3 Precision

13.3.1 The ability of the laboratory personnel to obtain consistent, precise results must be demonstrated with reference toxicants before attempts are made to measure the toxicity of sediment samples.

13.3.2 The single laboratory precision of the *Ampelisca* test should be determined by performing at least five or more preliminary tests with a reference toxicant. Short-term (*e.g.*, 96-hr) tests without sediments may be used for this purpose. Precision can be described by the mean LC50 (determined for each test by an appropriate method of regression analysis), standard deviation, and percent relative standard deviation (coefficient of variation, or CV) of the five (or more) replicate reference toxicant tests.

13.3.3 Because single laboratory precision has not been determined previously for the *Ampelisca* test, criteria for laboratory acceptance cannot be specified. Continual monitoring of reference toxicant test precision during the EMAP-Estuaries project, facilitated by the use of control charts (Subsection 13.5, Control Charts), will provide the basis for establishing such criteria.

13.4 Replication and Test Sensitivity

13.4.1 The sensitivity of the tests will depend in part on the number of replicates, the probability level selected, and the type of statistical analysis. The recommended minimum number of replicates and the statistical method(s) useful for addressing the study objectives are detailed in Subsection 14, Test Procedures for Marine Sediments Using *Ampelisca abdita*. In general, the number of replicates used in a test should be adequate for testing hypotheses and detecting departures from the assumptions of the particular statistical analyses employed.

13.5 Control Charts

13.5.1 A control chart should be prepared for each reference toxicant-organism combination, and successive toxicity values should be plotted and examined to determine if the results are within prescribed limits. In this technique, a running plot is maintained for the toxicity values (X_i) from successive tests with a given reference toxicant (Ziegenfuss et al., 1986). For regression analysis results (such as EC50s), the mean (X) and upper and lower control limits (± 2 standard deviations) are recalculated with each successive point until the statistics stabilize. Outliers, which are values which fall outside the upper and lower control limits, and trends of increasing or decreasing sensitivity are readily identified. At the $P_{0.05}$ probability level, one in twenty tests would be expected to fall outside of the control limits by chance alone.

13.5.2 If the toxicity value from a given test with the reference toxicant does not fall in the expected range for the test organisms, the sensitivity of the organisms and the overall credibility of the test are suspect.

In this case, the test procedure should be examined for defects and should be repeated with a different batch of test organisms.

13.6 Record Keeping and Reporting

13.6.1 Proper record keeping is required. Bound notebooks should be used to maintain detailed records of the test organisms such as species, source, age, date of receipt, and other pertinent information relating to their history and health, and information on the calibration of equipment and instruments, test conditions employed, and test results. Annotations should be made on a real time basis to prevent loss of information. Data for all QA/QC variables, such as reference toxicant test results and copies of control charts, should be submitted by the laboratory as part of the data package.

13.6.2 The record of the results of an acceptable sediment toxicity test should include the following information either directly or by reference to other available documents.

13.6.2.1 Names of test and investigator(s), name and location of laboratory, and dates of initiation and termination of the test.

13.6.2.2 Source and method of preparation of water used, its salinity and any other pertinent chemical characteristics.

13.6.2.3 Source of the control sediments, dates and methods of collection, method of transport and storage of field sediments, method and dates of treatment of laboratory-prepared sediment, and method of distribution to test chambers.

13.6.2.4 Source and date of collection of the test organisms, scientific name, method of taxonomic verification, age, life stage, means and ranges of lengths, observed diseases or unusual appearance, treatments, holding, and acclimation procedures.

13.6.2.5 Description of the experimental design, test chambers and covers, the depth and volume of sediment and water in the chambers, the date, time and method of beginning the test, numbers of test organisms and chambers, temperature, salinity, and lighting regime.

13.6.2.6 The average and range of holding and test temperatures, and the method(s) of measuring or monitoring or both.

13.6.2.7 Effects used to calculate EC50s (*e.g.*, fecundity, growth) and a summary of general observations of other effects.

13.6.2.8 A table of the biological data for each test chamber for each treatment (including the control(s)) in sufficient detail to allow independent statistical analyses.

13.6.2.9 The 10-day LC50s or EC50s and the methods used to calculate them, and their 95% confidence limits, or the survival or mortality data and their significance relative to the control(s); specify whether results are based on measured or nominal concentrations of the test material.

13.6.2.10 The mean, standard deviation, and range of the length measurements for the additional group of animals sorted and preserved at the beginning of each test.

13.6.2.11 Anything unusual about the test, any deviation from these procedures, and any other relevant information.

13.6.3 Published reports should contain enough information to clearly identify the procedures used and the quality of the results.

14. TEST PROCEDURE FOR MARINE SEDIMENTS USING *Ampelisca abdita*

14.1 Dissolved Oxygen (DO)

14.1.1 The concentration of dissolved oxygen (DO) in the water overlying the sediment in the test chambers should be maintained at or near saturation by gently aerating the water (see appendices). Air should be bubbled into the test chambers at a rate that maintains a $\geq$ 90% dissolved oxygen concentration, but does not cause turbulence or disturb the sediment surface. If air flow to the beakers is interrupted for more than an hour, DO should be measured in the beakers to determine whether dissolved oxygen concentrations have dropped to less than 60% of saturation.

14.2 Temperature

14.2.1 The temperature selected should be within the natural range of temperatures in the area from which the amphipods occur in the field. Within an experiment, individual temperature readings should not vary by more than 3°C from the selected test temperature, and the time-weighted average measured temperature at the end of the test should be within 1°C of the selected test temperature. When temperature is measured concurrently in more than one test chamber, the highest and lowest temperatures should not differ by more than 2°C.

14.2.2 This species is routinely tested at 20°C, but has been tested from 8 to 25°C. In nature, feeding and somatic growth occur at temperatures as low as 3 to 5°C (Bousfield, 1973). For comparison with other *Ampelisca abdita* test results, 20°C is recommended.

14.3 Salinity

14.3.1 The salinity of the water overlying the test sediment in sediment toxicity tests must be within the tolerance range of the selected test species.

14.3.2 *A. abdita* is tolerant of a wide salinity range, but most tests have been conducted at salinities of 28 to 32 ‰.

14.3.3 The salinity of the interstitial water of test sediments from the field should not be adjusted, because such an operation might change the toxicological properties of the sediment.

14.4 Light/Photoperiod

14.4.1 For sediment toxicity tests involving *A*. *abdita*, lights are usually left on continuously. The constant light increases the tendency of the organisms to remain buried in the sediment, and thus to remain exposed to the test material.

14.5 Feeding

14.5.1 Infaunal amphipods do not require supplementary feeding during the 10-day toxicity test.

14.6 Beginning the Test

14.6.1 The exposure chamber routinely used to test *A*. *abdita* is a 1-L glass canning jar with a narrow mouth.

14.6.2 *Ampelisca abdita* has not been tested in the 1-L beaker exposure chamber used in other amphipod tests, but it is not anticipated that use of beakers would create any problems.

14.6.3 This amphipod inhabits fine-grained sediments, and as with other physical conditions, if it is suspected that a coarse grain size of a test sediment will stress the animals, a grain size control should be included.

14.6.4 The toxicity test begins when test organisms are first placed in test chambers containing test material.

14.6.5 On the day before the test begins, each test sediment sample should be thoroughly homogenized within its storage container, and an aliquot added to a test chamber to a depth of 3 to 4 cm.

14.6.6 Treatments should be randomly assigned to prenumbered test chambers.

14.6.7 The sediment within the test chamber should be settled by tapping the test chamber against the side of the hand, or by smoothing the sediment surface with a nylon, fluorocarbon or polyethylene spatula.

14.6.8 A disk cut from 6-mil nylon, Teflon or polyethylene sheeting to fit the inside diameter of the test chamber, and attached to a length of nylon monofilament for removal, can be placed on the sediment surface to minimize sediment disruption as prepared toxicity test seawater is added up to the 800 ml mark on the test chambers.

14.6.8.1 The disk should be removed and rinsed with seawater between replicates of a treatment, and a separate disk should be used for each treatment. The test chambers should then be covered, put in numerical order into a temperature-controlled water bath, and aerated overnight. The system should be left overnight to allow suspended particles to settle and an equilibrium to be established between sediment and overlying water before the amphipods are added.

14.6.8.2 With either exposure chamber (1-L jar or beaker), the water column should be gently aerated with a pipette inserted above the sediment surface.

14.6.9 The toxicity test is initiated (Day 0) when amphipods are distributed to each test chamber. It is usually not possible to distribute amphipods to all test chambers at the same time, so it is necessary to select a set of test chambers (usually 10 to 15) to be processed together. If treatments are replicated, each treatment, including controls, should be represented in each set of test chambers to be processed together. If treatments are not replicated, selection should be random.

14.6.10 A sufficient number of amphipods should be removed from the holding facility at one time to provide about one-third more amphipods than are needed for one set of test chambers. This allows selection of active, apparently healthy individuals.

14.6.10.1 Before amphipods are removed, the temperature and salinity of the water in the holding containers should be recorded.

14.6.10.2 Amphipods should be sieved from the holding sediment using a 0.5 mm sieve and transferred to a sorting tray or large Carolina dish containing water of the holding temperature and salinity. Twenty to thirty amphipods should be tested per replicate.

14.6.10.3 Investigators who purchase amphipods from an outside source and have them shipped to the testing facility may prefer the following procedures for holding and selecting test amphipods, in lieu of the holding and sieving methods described above.

14.6.10.4 Amphipods are collected in the field while still remaining in their sediment mats. Disturbance to the mat is held to a minimum and the mat is packaged in clean, aerated seawater, then shipped to the testing facility via overnight mail.

14.6.10.5 Upon receipt at the testing facility, the mat containing the amphipods is immediately placed in chilled (20°C), aerated seawater of appropriate salinity to allow the amphipods to acclimate to test conditions for at least 48 hours prior to testing.

14.6.10.6 Amphipods are coaxed from the sediment mat by the simultaneous action of gently massaging a section of the mat while irrigating it with test dilution water to separate the amphipods from their tubes. The section of loosened mat is then placed onto a 0.5 mm sieve and submerged under water to rinse the amphipods clear of the mat. The amphipods tend to become trapped by the surface tension of the water from which they can easily be collected by scooping with a fine-mesh net or piece of Nitex screen. The captured amphipods are released into a sorting tray or large Carolina bowl containing sediment-free, test dilution water.

14.6.10.7 The isolated animals are then transferred to a lighted table for manual sorting using a small-bore pipet. Active, apparently healthy amphipods should be impartially selected from the sorting tray and sequentially distributed among dishes containing approximately 150 ml of prepared toxicity test seawater until each dish contains the required 20 to 30 immature amphipods. Amphipods should meet the following criteria to be selected for testing: a) amphipod should have a standard length of 3 to 5 mm when stretched out or 1 to 2 mm when curled; b) the gut of the animal should be full, as indicated by a dark line located dorsally on the animal; c) the animal must maintain a healthy appearance. While swimming, it is outstretched and searching for sediment or cover; at cessation of swimming it immediately curls up. Care should be taken not to select gravid females or males nearing sexual maturity. The number of amphipods in each dish should be verified by recounting them into a separate dish containing sediment-free, test dilution water.

14.6.10.8 At least one additional group of 20 to 30 amphipods should be randomly sorted at the beginning of each test. This extra group should be preserved in 5 to 10% buffered formalin for later length measurement as a check to ensure that appropriately-sized amphipods were selected for testing. Length of each individual in the group should be determined, using a dissecting microscope, by measuring from the base of the first antennae to the base of the telson. The measurements (mean length, standard deviation, and range) should be recorded on the data sheets for each test and reported along with the final results.

14.6.11 Amphipods should be added to test chambers by placing a 6-mil nylon, Teflon or polyethylene disk on the water surface, and gently pouring the water and amphipods from the sorting dish over the disk into the test chamber. An alternative method would be to gently pour the organisms onto a Nitex screen and then gently wash them into the test chamber.

14.6.11.1 For each replicate, the contents of a sorting dish can be rinsed into a plastic cup with a 400- or 500-μm screened base and then into the exposure container. Any animals caught on the water's surface can be gently pushed under using a glass rod. Any amphipods remaining in the dish should be gently washed into the test chamber.

14.6.11.2 The water level should be brought up to the final test level in the test chamber (800 ml), the disk removed, and the chamber replaced in the water bath, covered and aerated.

14.6.12 Amphipods should be given one hour to burrow into the sediment. Any amphipods which do not burrow within one hour should be removed and replaced.

14.7 Routine Chemical and Physical Analysis

14.7.1 Monitoring the quality of the overlying water (for DO, pH, or for certain chemicals) in the test chambers can be accomplished without disturbing the sediment, and may be done in the test chambers containing the test amphipods.

14.7.2 Temperature from a separate temperature beaker should be recorded throughout the test. If test chambers are in more than one temperature controlled water bath, a temperature beaker should be set up in each water bath. Temperature should be monitored at least hourly using a recording thermometer or the daily maximum and minimum temperatures should be monitored (see ASTM Standard E- 729). Individual temperature measurements should not vary by more than 3°C and the time-weighted average should not differ by more than 1°C from the designated test temperature (see Subsection 13.2, Temperature).

14.8 Duration of Test

14.8.1 The test begins when amphipods are added to test chambers containing test sediment. Amphipods should be exposed to the test material for ten days. There are no observed substantial effects of starvation or other laboratory artifacts in this amount of time (Swartz *et al*., 1985). An exposure period of less than ten days is generally not recommended. For some experimental designs, such as comparison of a 96-hr LC50 between species in the presence or absence of sediment, other exposure periods may be used.

14.9 Observations During the Test

14.9.1 Response criteria indicating toxicity of test sediment include mortality and sublethal effects. Sublethal effects include an emergence from highly toxic sediment during the course of the test. Response criteria must be monitored in a "blind" fashion, that is, the observer must have no knowledge of the treatment of the sediment in the test chambers. This is accomplished through randomization of sample numbers.

14.9.2 Emergence - Since most infaunal amphipods remain buried during sediment toxicity tests, there is little opportunity to monitor temporal changes in mortality or sublethal effects. An exception is the temporal pattern of emergence from highly toxic sediment.

14.9.3 The test should be monitored at least daily (including the day of initiation and the day of termination) for temperature, aeration, lights, and emergence of the amphipods from the test sediment.

14.9.3.1 Each test chamber should be observed by temporarily turning off the air to the test chambers, and gently removing the cover from individual chambers with minimal disturbance of the chamber.

14.9.3.2 The number of amphipods observed completely or partially out of the sediment, either on the sediment surface, swimming in the overlying water, or floating at the water surface, should be recorded. Amphipods that are caught in the surface film should be gently pushed down into the water.

14.9.3.3 Any pertinent observations on the appearance of the sediment (such as color, presence of non-test organisms, growth of mold or algae, or depth of oxidized layer) should be recorded.

14.10 Test Termination

14.10.1 The endpoint for the 10-day test is mortality; dead animals should be counted and removed daily. An amphipod is considered dead if it does not respond to gentle probing. It is also useful to note any animals out of their tubes on the sediment or water surface, amphipods which are nearly dead and only exhibit a muscular pleopod twitch, the presence of molts, and the condition of the tubes built. Emergence from the sediment and the inability to construct a proper tube are sublethal behavioral responses that would ultimately result in death.

14.10.2 After checking the assay on the last day, the contents of each exposure container should be rinsed through a 0.5-mm sieve. (A smaller mesh sieve can be used for the final sieving if there is concern about losing very small animals, but this will make the sieving process more time-consuming.)

14.10.3 If the experiment is small, the material retained on the sieve can be examined that day. If time does not permit same-day examination, the retained material from each jar can be preserved in 5% buffered formalin with Rose Bengal stain for later examination.

14.10.4 Any amphipods which are not accounted for when the sieved material is examined are presumed to have died during the test. Amphipods which have died in their tubes will generally decompose during the test or break apart during sieving. Rarely, an individual which has died during the test will be recovered in the preserved material, and its appearance will be markedly different from those of the amphipods which were alive when preserved. For instance, there may be little tissue left within its exoskeleton, or it may be contorted.

14.10.5 If the test species is naturally present in the test sediment, the total number of live and dead amphipods at Day 10 might exceed the number at Day 0.

14.11 Acceptability of Test

14.11.1 A 10-day sediment toxicity test should usually be considered unacceptable if one or more of the following occurred:

14.11.1.1 All test chambers were not identical.

14.12.1.2 Treatments were not randomly assigned to test chambers.

14.11.1.3 Test organisms were not randomly or impartially distributed to test chambers.

14.11.1.4 Required negative, reference sediment, positive or solvent controls were not included in the test.

14.11.1.5 All test animals were not from the same population, were not all of the same species, or were not of acceptable quality.

14.11.1.6 The average length of the animals used in the test was not within the range 3 to 5 mm, or there were wide variations in length.

14.11.1.7 Amphipods from a wild population were maintained in the laboratory for more than ten days, unless the effects of prolonged maintenance in the laboratory has been shown to have no significant effect on sensitivity.

14.11.1.8 The test organisms were not acclimated at the test temperature and salinity at least 48 hours before they were placed in the test chambers.

14.11.1.9 Temperature, dissolved oxygen and concentration of test material were not measured, or were not within the range as specified in Section 14.7, Routine Chemical and Physical Analysis.

14.11.1.10 Aeration to the test chambers was off for an extended time such that dissolved oxygen levels dropped to less than 60% of saturation.

14.11.1.11.2 Response criteria were not monitored in a "blind" fashion, *i.e*., observers had knowledge of the treatment of sediments in the test chambers.

14.11.2 Survival of organisms in control treatments should be assessed during each test as an indication of both the validity of the test and the overall health of the test organism population. Tests are acceptable if mean control survival is greater than or equal to 90%, and if survival in individual control test chambers exceeds 80%.

15. TEST PROCEDURE FOR MARINE SEDIMENTS USING MYSIDS (*Mysidopsis bahia*) AND PENAEID SHRIMP

15.1 Dissolved Oxygen (DO)

15.1.1 The concentration of dissolved oxygen (DO) in the water overlying the sediment in the test chambers should be maintained at or near saturation by gently aerating the water (see appendices). Air should be bubbled into the test chambers at a rate that maintains a $\geq$ 90% DO concentration, but does not cause turbulence or disturb the sediment surface. If air flow to the beakers is interrupted for more than an hour, DO should be measured in the beakers to determine whether dissolved oxygen concentrations have dropped to less than 60% of saturation.

15.2 Temperature

15.2.1 The sediment tests with mysids and penaeids will be run at a temperature of 20^{o}C. Within an experiment, individual temperature readings should not vary by more than 3^{o}C from this test temperature, and the time-weighted average measured temperature at the end of the test should be within 1^{o}C of this test temperature. When temperature is measured concurrently in more than one test chamber, the highest and lowest temperatures should not differ by more than 2^{o}C.

15.3 Salinity

15.3.1 The EMAP-Estuaries tests with mysids and penaeids will be conducted using natural, filtered ($\leq$ 20 μm) seawater with the salinity maintained at 20 $\pm$ 2 ‰. No attempt will be made to adjust the salinity of the interstitial water of the test sediments.

15.4 Light/Photoperiod

15.4.1 In sediment toxicity tests involving mysids and penaeids, a 14-hour light and 10-hour dark photoperiod is established with cool-white fluorescent lights adjusted to approximately 1200 microwatts/cm^2.

15.5 Test Organisms

15.5.1 Mysids. Newly released juveniles are collected by siphoning water from holding tanks through nylon screen chosen to allow only the passage of juveniles which are concentrated in a small mesh cage. Juveniles are transferred to holding tanks where gradual acclimation to test conditions occurs. Within a 24-hour period, changes in water temperature should not exceed 5°C and salinity changes should not exceed 5‰. During acclimation, mysids should be maintained in facilities with background colors and light intensities similar to those of the testing areas. *Mysidopsis bahia* must be 3 to 5 days old and all animals for any one test must be from the same source; that is, animals cultured in the laboratory and purchased from a supplier cannot be mixed to form a test population for a sediment or reference toxicant test. Whenever possible, mysids to be used in toxicity tests should originate from the laboratory to ensure the individuals are of similar age and known history.

15.5.2 Penaeids (sp). Post-larval or juvenile shrimp should be used. Shrimp may be reared from eggs in the laboratory or obtained directly from the field as juveniles. As with mysids, all animals for any one test must be from the same source. Shrimp to be used in a particular test should be of similar age and be of normal size and appearance.

15.5.3 Upon arrival at the test facility, the juvenile penaeid shrimp should be transferred to water closely matching the temperature and salinity of the transporting medium. The period of acclimation to ambient laboratory conditions should be at least 4 to 7 days. During acclimation, shrimp should be maintained in facilities with background colors and light intensities similar to those of the testing areas. In addition, any change in the temperature and chemistry of the dilution water used for holding and acclimating the test organisms to those of the test should be gradual. Within a 24-hour period, changes in water temperature should not exceed 2°C, and salinity changes should not exceed 5‰.

15.6 Feeding

15.6.1 Mysids and penaeid post larvae will be fed artemia (24 to 48 hours post hatch) once a day (approximately 100 artemia nauplii per animal per day). Juvenile penaeids will not be fed during the 96-hour test duration.

15.7 Test Material

15.7.1 All samples will be chilled during shipment to the laboratory. Immediately after receipt, the sediment sample(s) must be placed in a cooler and maintained at 4±1°C. A record of temperature during storage must be provided as part of the report.

15.8 Glassware

15.8.1 All glassware is detergent-washed, rinsed thoroughly with tapwater, rinsed with acetone, rinsed thoroughly with deionized water, soaked in 10% HCl for four hours, and rinsed thoroughly with tapwater and five times with deionized water.

15.9 Beginning the Test

15.9.1 The exposure chambers for the mysid and post larval penaeid test will be 1-L beakers. The exposure chambers for the juvenile penaeid test (if post larvae are not available) will be 2-L Carolina dishes.

15.9.2 As the sample arrives, each entire test sediment sample should be press-sieved (dry) through a stainless steel screen (1.0-mm mesh size) to remove predators and larger particles (*e.g.*, rocks, shells).

15.9.3 On the day before the test begins each sediment sample is homogenized using a high speed (≥ 1600 rpm) stirrer. Sample should freely "roll" while being stirred and all sediment from sides and bottom of sample container should be mixed into the sample.

15.9.4 The sediment within the test chamber should be settled by tapping the test chamber against the side of the hand, or by smoothing the sediment surface with a polyethylene spatula.

15.9.5 A petri dish is placed on the sediment surface to minimize sediment disruption as prepared toxicity test seawater is added up to the 700-ml mark in the test chambers.

15.9.6 The petri dish should be removed and rinsed with seawater between replicates of a treatment, and a separate dish should be used for each treatment. The test chambers should then be covered, labeled, transferred into a temperature-controlled water bath or environmental chamber where the tests shall be conducted and aerated overnight. The system should be left overnight to allow suspended particles to settle and an equilibrium to be established between sediment and overlying water before the test animals are added.

15.9.7 The water column should be gently aerated with a glass pipette inserted above the sediment surface.

15.9.8 The toxicity test is initiated (Day 0) when mysids or penaeids are distributed to each test chamber. Three replicate test chambers per test organism will be used for each test sediment.

15.9.9 Before mysids and penaeids are removed from the holding containers, the temperature and salinity of the water in the holding containers should be recorded. A sufficient number of mysids and penaeids should be removed from the holding facility at one time to provide about one-third more animals than are needed for the test chambers.

15.9.10 The mysids or penaeids should be transferred to a sorting tray containing water of the holding temperature and salinity. Active, apparently healthy mysids or penaeids should be impartially selected from the sorting tray and sequentially distributed among intermediate holding dishes containing approximately 150 ml of prepared toxicity test water until each dish contains the required number of ten animals.

15.9.11 The animals in the intermediate holding dish should be counted to verify the number of animals to be loaded into the testing chamber and observed to be sure the animals are active and healthy. The water in the holding dish is then drawn down to a small volume before the entire lot is transferred to a test chamber.

15.9.12 The test animals should be impartially distributed among test chambers in such a manner that test results show no significant bias from the distributions. The test chambers are then positioned randomly.

15.10 Routine Chemical and Physical Analysis

15.10.1 Monitoring the quality of the overlying water in the test chambers can be accomplished without disturbing the sediment, and may be done in the test chambers containing the test animals.

15.10.2 Temperature, from a separate temperature beaker, should be recorded throughout the test. If test chambers are in more than one temperature-controlled water bath or environmental chamber, a temperature beaker should be set up in each. Temperature should be monitored at least hourly using a recording thermometer or the daily maximum and minimum temperatures should be monitored (see ASTM Standard E 729). Individual temperature measurements should not vary by more than 3°C and the time-weighted average should not differ by more than 1°C from the designated test temperature.

15.11 Duration of Test

15.11.1 The test begins when mysids or penaeids are added to the test chambers containing the test sediment. Mysids and penaeids should be exposed to the test material for 96 hours.

15.12 Observations During the Test

15.12.1 Mortality and lethargic behavior are the responses that will be monitored during the sediment toxicity tests with mysids or penaeids.

15.12.2 The test should be monitored at least daily (including the day of initiation and the day of termination) for temperature, aeration, lights, and overall behavior of the mysids or penaeids exposed to the test sediments.

15.12.3 Each test chamber should be observed by temporarily turning off the air to the test chambers, and gently removing the cover from individual chambers with minimal disturbance of the chamber.

15.12.4 The number of mysids or penaeids observed on the sediment surface, swimming in the overlying water, or floating at the water surface should be recorded. Mysids or penaeids that are caught in the surface film should be gently pushed down into the water.

15.12.5 Water quality parameters (*i.e*., dissolved oxygen and pH) will be measured in at least one replicate of each test treatment and control on Day 0 and Day 4 of testing.

15.12.6 Any pertinent observations on the appearance of the sediment (such as color, presence of non-test organisms, growth of mold or algae, or depth of oxidized layer) should be recorded.

15.13 Test Termination

15.13.1 The endpoint for the 96-hour mysid and penaeid test is mortality; dead animals should be counted and removed daily. A mysid or penaeid is considered dead if it does not respond to gentle probing.

15.13.2 After checking the assay on the last day, the contents of each exposure container should be rinsed through a 0.5 mm sieve.

15.13.3 If the experiment is small, the material retained on the sieve can be examined that day. If time does not permit same-day examination, the retained material from each jar can be preserved in 5% buffered formalin with Rose Bengal stain for later examination.

15.13.4 Any mysids or penaeids which are not accounted for when the sieved test material is examined are presumed to have died during the test.

15.14 Acceptability of Test

15.14.1 A 96-hour sediment toxicity test should usually be considered unacceptable if one or more of the following occurred:

15.14.1.1 All test chambers were not identical.

15.14.1.2 Test organisms were not randomly or impartially distributed to test chamber.

15.14.1.3 A required control treatment consisting of clean sediment was not included in the test.

15.14.1.4 All test animals were not from the same population, were not all of the same species, or were not of acceptable quality.

15.14.1.5 The test organisms were not acclimated to within ± 5° C of the specified test temperature and ± 5‰ of the specified test salinity at least 24 hours before they were placed in the test chambers.

15.14.1.6 Temperature, dissolved oxygen and concentration of test material were not measured, or were not within acceptable ranges.

15.14.1.7 Aeration to the test chambers was off for an extended time such that dissolved oxygen levels dropped to less than 60% of saturation.

15.14.1.8 Survival of organisms in control treatments should be assessed during each test as an indication of both the validity of the test and the overall health of the test organism population. Tests are acceptable if mean control survival is greater than or equal to 90% and if survival in individual control test chambers exceeds 80%.

16. CALCULATIONS

16.1 The calculating procedure(s) and interpretation of the results should be appropriate to the experimental design.

16.2 Procedures used to calculate results of toxicity tests can be divided into two categories: those that test hypotheses and those that provide point estimates.

16.3 No procedure should be used without careful consideration of a) the advantages and disadvantages of various alternative procedures and b) appropriate preliminary tests, such as those for outliers and for heterogeneity. Preprocessing of data may be required to meet the assumptions of the analyses.

16.4 LC50 or EC50 and their 95% confidence limits should be calculated based on (a) the measured initial concentrations of test material, if available, or the calculated initial concentrations, and (b) percent mortality. If other LCs or ECs are calculated, their 95% confidence limits should also be calculated (see ASTM Practice E 729).

16.5 Most acute toxicity tests produce quantal data, *i.e.*, counts of the number of organisms in two mutually exclusive categories, such as alive or dead.

16.5.1 A variety of methods (Litchfield and Wilcoxon, 1949; Finney, 1964; Finney, 1971; Stephan, 1977; Hamilton *et al*., 1977) can be used to calculate an LC50 or EC50 and its 95% confidence limits from a set of quantal data that is binomially distributed and contains two or more concentrations at which the percent dead or affected is between 0 and 100, but the most widely used are the probit, moving average, trimmed Spearman-Karber and Litchfield-Wilcoxon methods.

16.5.2 The method used should appropriately take into account the number of test chambers per treatment and the number of test organisms per chamber. The binomial test can usually be used to obtain statistically sound information about the LC50 or EC50 even when less than two concentrations kill or affect between 0 and 100 percent.

16.5.3 The binomial test does not provide a point estimate of the LC50 or EC50, but it does provide a range within which the LC50 or EC50 should lie.

16.6 The results of toxicity tests on field samples without replication may be reported in terms of survival values. A sample should be considered to be toxic if the single sample value lies outside the 95% tolerance limits of the survival of the controls.

16.6.1 Alternately, the field result may be compared with the control survival data using outlier detection methods; the sample may be considered toxic if it would be rejected as an extreme value when considered as part of the control population.

16.6.2 Another approach is to use the "special case" comparison of a single value against a sample, described by Sokal and Rohlf. It is strongly recommended that samples be replicated if comparisons among sites are desired.

16.7 If samples from field stations are replicated, the mean survival at the stations and the mean control survival should be statistically compared by a one-tailed t-test or analysis of variance (ANOVA) followed by a multiple comparison test.

16.7.1 Analysis of variance is used to determine whether any of the observed differences among the concentrations (or samples) are statistically significant. This is a test of the null hypothesis of no difference among concentrations (or samples).

16.7.2 If the F-test is not statistically significant ($P>0.05$), it can be concluded that the effects observed in the toxicant treatments (or field station samples) were not large enough to be detected as statistically significant by the experimental design and hypothesis test used.

16.7.3 Following a significant F-test result, all exposure concentration effects (or field station samples) can be compared with the control effects by using mean separation techniques such as those explained by Chew (1977) orthagonal contrasts, Fisher's methods, Dunnett's procedure and Williams' method.

16.8 The Dunnett's procedure is a multiple comparison test specifically designed to compare several experimental samples to the concurrent control (Gelber *et al*., 1985). A multiple comparison test is a technique that accounts for the fact that several comparisons are being made simultaneously.

16.9 Daily observations on the number of amphipods which have completely or partially emerged from the sediment, either lying on the sediment surface, swimming in the water column, or floating at the water surface, can be used to document an apparent avoidance response to the sediment. Emergence data

plotted against time can give the observer an impression of the degree of toxicity of the sediment during the course of the toxicity test, as amphipods often emerge earlier and in greater numbers from more highly toxic sediment.

APPENDIX A

DILUTION/CULTURE WATER FOR *Ampelisca abdita*

A.1 GENERAL REQUIREMENTS

A.1.1 Besides being available in adequate supply, water used in toxicity tests should be acceptable to test organisms and the purpose of the test. The minimum requirement for acceptable water for use in acute toxicity tests is that healthy test organisms survive in the water, and in the water with sediment for the duration of holding and testing without showing signs of disease or apparent stress such as unusual behavior, changes in appearance, or death. The water in which the test organisms are held prior to the test should be uniform in quality in that the concentration of contaminants and the range of temperature and salinity encountered during the holding period do not adversely affect the survival of the test organisms in the holding tanks or in the control treatments during the test.

A.2 SOURCE

A.2.1 Natural Salt Water - If natural salt water is used, it should be obtained from an uncontaminated area known to support a healthy, reproducing population of the test species or a comparably sensitive species. The water intake should be positioned to minimize fluctuations in quality and the possibility of contamination, and to maximize the concentration of dissolved oxygen to help ensure low concentrations of sulfide and iron. A specially designed system might be necessary to obtain salt water from a natural water source. These precautions are intended to ensure that test organisms are not apparently stressed by water quality during holding, acclimation and testing and that water quality does not unnecessarily affect test results.

A.2.2 Reconstituted Salt Water - Reconstituted salt water can be prepared by adding a commercially available sea salt or specified amounts (Table 1, see ASTM Standard E 729) of reagent-grade chemicals (American Chemical Society, 1968) to high quality water with (a) conductivity less than 1 µS/cm and (b) either total organic carbon (TOC) less than 2 mg/L or chemical oxygen demand (COD) less than 5 mg/L. Acceptable water can usually be prepared using properly operated deionization or distillation units. Reconstituted salt water should be intensively aerated before use, and aging for one to two weeks might be desirable. If a residue or precipitate is present, the solution should be filtered before use. The water should meet the criteria given in Subsection A.1.1.

A.2.3 Chlorinated water must never be used in the preparation of water for toxicity tests because residual chlorine and chlorine-produced oxidants are highly toxic to many aquatic animals (U.S. EPA 1985). Dechlorinated water should be used only as a last resort because dechlorination is often incomplete. Municipal drinking water is not recommended for use because in addition to residual chlorine,it often contains unacceptably high concentrations of metals, and quality is often highly variable (see ASTM Standard E 729).

A.3 PREPARATION

A.3.1 Seawater used in the sediment toxicity test should be passed through a filter effective to 5 µm or less to remove suspended particles and organisms from the water. Water that might be contaminated with facultative pathogens should be passed through a properly maintained ultraviolet sterilizer (National Academy of Sciences and National Academy of Engineering, 1973; Federal Register, 1980; Federal Register, 1985) or a filter effective to 0.45 µm or less.

A.3.1.1 If necessary, the salinity should be reduced by diluting the seawater with high quality deionized or distilled water (see Subsection A.2.2). Salinity can be raised by addition of clean filtered oceanic water or prepared brine. Common practice is to use a 60 to 90 ‰ saltwater brine. Such brines have been successfully prepared using slow, heat-concentration of natural salt water, or by the addition of artificial sea salts or reagent grade (Americal Chemican Society, 1968) salts to a natural salt water (see Subsection A.2.2).

A.3.2 Fresh seawater used in the test should be prepared within two days of the test and stored in clean, covered containers at 4 ± 3°C until sediment and water are added to the test chambers. It might be necessary to age reconstituted seawater for one to two weeks before use. Sufficient water should be prepared at one time for all test chambers. Additional water might be required for sieving control sediment to adjust salinity or for holding the test amphipods prior to the test.

A.3.3 For certain applications the experimental design might require use of seawater from the test sediment collection site. In other instances, experimental treatments might involve manipulation of the test seawater conditions.

TABLE 1. Reconstituted Salt Water (Kester *et al.*, 1967; Zaroogian *et al*., 1969; Zillioux *et al.*, 1973) for marine and estuarine crustaceans. Add the following reagent-grade (American Chemical Society, 1968) chemicals in the amounts and order listed to 890 ml of water. Each chemical must be dissolved before the next is added.[A]

Chemical	Amount
NaF	3 mg
$SrCl_2 \cdot 6H_2O$	20 mg
H_3BO_3	30 mg
KBr	100 mg
KCl	700 mg
$CaCl_2 \cdot 2H_2O$	1.47 g
Na_2SO_4	4.00 g
$MgCl_2 \cdot 6H_2O$	10.78 g
NaCl	23.50 g
$Na_2SiO_3 \cdot 9H_2O$	20 mg
$NaHCO_3$	200 mg

[A] If the resulting solution is diluted to 1 L, the salinity should be 34 ± 0.5 ‰ and the pH 8.0 ± 0.2. The desired test salinity is attained by dilution at time of use. The reconstituted salt water should be stripped of trace metals (Davey *et al*., 1970).

APPENDIX B

TEST ORGANISM: *Ampelisca abdita*

B.1 ECOLOGY

B.1.1 *Ampelisca abdita* is a tube-dwelling amphipod belonging to the family Ampeliscidae, found mainly in protected areas from the low intertidal zone to depths of 60 m. It ranges from central Maine to south-central Florida and the eastern Gulf of Mexico (Bousfield, 1973; Mills, 1964), and has also been introduced into San Francisco Bay (Nichols and Thompson, 1985). It is euryhaline, and has been reported in waters which range from fully marine to 10‰ salinity (Mills, 1967). This species generally inhabits sediments from fine sand to mud and silt without shell, although it may also be found in relatively coarser sediments with a sizable fine component (Mills, 1967). *A. abdita* is often abundant in sediments with a high organic content (Santos and Simon, 1980**)**.

B.1.2 In the colder waters of its range, *A. abdita* produces two generations per year, an overwintering generation which breeds in the spring and a second which reproduces in mid to late summer (Nichols and Thompson, 1985; Mills, 1967). In New England, breeding of the overwintering generation begins when the water temperature is about 8°C, but in warmer waters south of Cape Hatteras, breeding may be continuous throughout the year. Adults mate in the water column, and intense breeding activity is correlated with the full moon and spring tides. Juveniles are released after approximately two weeks in the brood pouch, at about 1.5mm in length. It then takes 40 to 80 days for newly released juveniles to become breeding adults (Mills, 1967). Where *A. abdita* are present, they are often dominant members of the benthic community with densities up to 110,000 m^{-2} (Nichols and Thompson, 1985; Stickney and Stringer, 1957; Santos and Simon 1980). *Ampelisca abdita* is a particle feeder, feeding both on particles in suspension and on those from the surface of the sediment surrounding its tube. Gut contents of field-collected specimens have been found to include algal material, sediment grains, and organic detritus (Bousfield, 1973; Nichols and Thompson, 1985).

B.2 COLLECTION AND HANDLING TECHNIQUES

B.2.1 *Ampelisca* should be sieved from their native (collection site) sediment as soon as possible after collection. A 2-mm mesh sieve nesting over a 0.5-mm mesh sieve is useful for this procedure. It is desirable for the sediment containing the amphipods to be rinsed first through the upper, 2-mm, sieve with a forceful stream of seawater at the collection temperature and salinity. This will break up the sediment material and will also force most of the amphipods out of their tubes.

B.2.1.1 The material thus retained on the 0.5-mm sieve should be vigorously shaken and swirled so the fine sediments pass through and the amphipods are separated from tubes, sediment, and detrital material. If the sieve is then lifted from the water, allowed to drain, and then slowly lowered into a shallow tray of seawater, the *Ampelisca* will be caught on the water's surface tension and can be easily collected with a fine mesh dip net.

B.2.1.2 The amphipods can be held temporarily in large culture dishes in a constant temperature bath, and then separated into two size classes with the use of nested 1.0- and 0.5-mm sieves.

B.2.2 During acclimation, 300-350 *Ampelisca* can be held in 4-L jars, each containing approximately a 4-cm deep layer of sieved collection site sediment. If seawater is flowing through the holding containers, a screened overflow must be used to prevent loss of swimming amphipods.

B.2.3 Amphipods should have food available on a daily basis during acclimation. Research is currently being conducted to determine optimal food sources for culturing this amphipod. Reasonable growth and reproduction have been obtained when *A. abdita* has been fed the diatom *Phaeodactylum tricornutum* daily in excess (a suggested amount is 0.5 to 1 L of algae per 4-L jar, or 14 X 10^6 cells/ml (U.S. EPA, 1994)). *Skeletonema costatum* has also been used successfully. Amphipod exposure to the food source will be increased if, during the feeding period (*e.g.*, overnight), the holding system is static, with aeration to circulate the algae. Sloping upper sides on the holding containers will aid in movement of algae across the sediment surface. Photoperiod should be maintained at 16 h light: 8 h dark.

B.2.4 Care should be taken to maintain the temperature with a water bath when seawater is not flowing through the jars. Approximate density in the holding jars should not exceed 300 amphipods (or one amphipod per cm^2 surface area). Acclimation to the test temperature should not exceed 3°C per day, amphipods should be used within ten days after collection, and they must be acclimated to laboratory conditions for at least 48 hours.

B.2.5 *Ampelisca abdita* may be shipped if this is done within one day of collection. Small plastic "sandwich" containers (approximately 500 ml) can be used to hold the amphipods. The containers are filled three-quarters full with a minimum depth of 2 cm of sieved collection site sediment and then to the top with well-aerated seawater.

B.2.6 No more than 200 amphipods should be added to each container. Amphipods should be allowed to burrow into the sediment and build tubes before the containers are capped. The capping must be done underwater to eliminate any air pockets in the containers. Containers should be shipped via overnight delivery in coolers with a few ice packs to prevent extreme temperature changes during transit.

B.3 OTHER TESTING WITH *Ampelisca abdita*

B.3.1 Growth of *Ampelisca abdita* has been measured in 10-day tests; the amphipods must be fed during the test. Small juveniles in a narrow size range (between 2 and 4 mm in length) should be selected for testing, and when sorting for the initiation of the test at least one additional group of amphipods should be sorted. This extra group represents the initial size and should be preserved in 5% buffered formalin for later measurement at a convenient time. Using a dissecting microscope, length is measured from the base of the first antennae to the base of the telson.

B.3.2 Chronic tests have also been conducted with this species (Scott and Redmond, 1989) and research is underway to determine the optimum conditions for those tests.

APPENDIX C

TEST ORGANISM: *Mysidopsis bahia*

C.1 NATURAL HISTORY

C.1.1 The estuarine species *Mysidopsis bahia*, a small (adults, 5 to 8 mm, total length) shrimp-like crustacean, belongs to the order Mysidacea and is frequently found along the Gulf Coast from southern Florida (Everglades) to Mexico (San Geronimo). Mysids are found in most bays and estuary systems which range in salinity from 8-25‰. They may be collected adjacent to tidal marshes or among marsh plants (*Juncus* or *Spartina*) during high tides.

C.1.2 The relative ease with which mysids can be cultured is a contributing factor to their routine use as an indicator organism. The sexes are separate and externally dimorphic. When sexually mature, females are easily identified with the use of magnification by their brood pouches. Through a dissecting microscope, males can be identified by the presence of claspers.

C.2 HANDLING AND CULTURING TECHNIQUES

C.2.1 Holding tanks Temperature-controlled, all-glass aquaria with flow-through or recirculating seawater maintained at 25 $\pm$ 2°C.

C.2.2 Aeration Oil-free forced air.

C.2.3 Light A 14-hour light and 10-hour dark photoperiod is established with cool-white fluorescent lights adjusted to approximately 1200 microwatts/cm^2.

C.2.4 Water Natural, filtered ($\leq$ 20 μm) seawater or artificial seawater formulated by the addition of sea salts to deionized water and adjusted to a salinity of 20 to 25‰.

C.2.5 Mysids are fed live *Artemia salina* nauplii at the rate of approximately 100 *Artemia* nauplii per mysid per day; the *Artemia* nauplii must be $\leq$ 36 hours old.

C.2.6 To provide an adequate supply of juveniles for a test, mysid cultures should be started at least four weeks before the test animals are needed. At least 200 mysids should be placed in each culture tank (a 2:1 female to male ratio) to ensure 1000 animals will be available by the time preparations for the test are initiated.

C.2.7 Newly released juveniles are collected by siphoning water from holding tanks through nylon screen chosen to allow only the passage of juveniles which are concentrated in a small mesh cage. Juveniles are transferred to holding tanks where gradual acclimation to test conditions occurs. Within a 24-hour period, changes in water temperature should not exceed 2°C, while salinity changes shall not exceed 5 ‰). During acclimation mysids should be maintained in facilities with background colors and light intensities similar to those of the testing areas.

APPENDIX D

TEST ORGANISM: *Penaeus* SPP.

D.1 NATURAL HISTORY

D.1.1 Penaeid larvae develop at sea and appear to be most active at night, moving vertically through the water column. Once they have reached the post-larval stage and migrated into the nursery areas, the animals become totally benthic and remain active both day and night. Penaeids are omnivorous, eating zooplankton, fragments of higher plants, and ingesting algae as well as sand, mud and organic debris.

D.1.2 Some penaeids can be laboratory cultured, but not with ease. Spawning can be induced in the laboratory by artificially stimulating egg production through the coupling of regulated lighting and temperature with eye stalk oblation of adult females.

D.2 HANDLING TECHNIQUES AND HOLDING CONDITIONS FOR POST-LARVAE OR JUVENILES

D.2.1 Holding tanks Temperature-controlled, all-glass aquaria with flow-through or recirculating seawater maintained at 25 $\pm$ 2°C.

D.2.2 Aeration Oil-free forced air.

D.2.3 Light A 12-hour light and 12-hour dark photoperiod is established with cool-white fluorescent lights adjusted to approximately 1200 microwatts/cm^2.

D.2.4 Water Natural, filtered ($\leq$ 20 μm) seawater or artificial seawater formulated by the addition of seasalts to deionized water and adjusted to a salinity of 20 to 25‰.

D.2.5 Penaeid post-larvae will be fed *Artemia*. Juveniles will be fed commercial fish flake.

D.2.6 Laboratory-reared, post-larvae will be generated several weeks prior to the initiation of the test and maintained at the laboratory facility.

D.2.7 Post-larval batches of penaeids will be segregated by age and used for Louisianian Province ITE samples. These samples will arrive randomly throughout the testing period.

D.2.8 If laboratory-reared post-larvae are not available, laboratory-reared juveniles or wild-caught *Penaeus* sp. will be used after an acclimation period of at least four days.

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SECTION 3

BENTHIC MACROINVERTEBRATE METHODS
MACROBENTHIC COMMUNITY ASSESSMENT

TABLE OF CONTENTS

Subsections

1. INTRODUCTION

1.1 This chapter describes the laboratory procedures used by the EMAP Estuaries Resource Group to measure the species composition, abundance, and biomass of macroinvertebrate fauna found in estuarine sediments.

1.2 Estuarine bottom-dwelling (benthic) assemblages have many attributes that potentially make them reliable and sensitive indicators of ecological condition and pollution stress (Boesch and Rosenberg 1981; Bilyard 1987). First, benthic organisms have limited mobility and cannot avoid exposure to pollutants and adverse conditions. Second, benthos live in bottom sediments where chemical contaminants accumulate and stress from low dissolved oxygen is most severe (*e.g.,* Mirza and Gray 1981; Chapman *et al*. 1987). Third, benthic assemblages are taxonomically diverse and include multiple feeding modes and trophic levels. As a result, they display a broad range of physiological tolerances and responses to multiple types of stress (Pearson and Rosenberg 1978; Rhoads *et al*. 1978; Sanders *et al.* 1980; Boesch and Rosenberg 1981; Swartz *et al.* 1986). Finally, benthic organisms are important links between primary producers and higher trophic levels including economically important species (Virnstein 1977; Holland *et al.* 1980), and their feeding and burrowing activities affect oxygen, carbon, nutrient, and sediment cycles (Rhoads and Young 1970; Rhoads 1974; Cloern 1982).

1.3 The procedures outlined in this chapter are designed to produce data of consistent quality meeting the measurement quality objectives (MQO) of 10% total error for the extraction of organisms from sediments and 10% total error for the identification and enumeration of extracted fauna. To ensure that these objectives are met, specific quality assurance steps are included as part of these procedures.

1.4 Laboratory procedures are based upon currently accepted practices in benthic ecology (Holmes and McIntyre 1984). Changes are not permitted without prior approval from the EMAP-Estuaries Technical Director. Such changes will be incorporated into subsequent versions of this manual in a timely manner.

1.5 The fixative for EMAP benthic samples will be formalin in all cases. Samples will be preserved in a 10% formalin solution for at least one month prior to processing. Subsequent preservation in a 70% ethanol solution is recommended.

1.6 These procedures assume that benthic samples arrive at the laboratory in pre-labeled, plastic screw-top jars, preserved in a rose bengal stained buffered, 10% formalin solution. Procedures used to prepare benthic samples in the field are given in the appropriate EMAP Estuaries Field Operations Manual and will not be repeated here.

2. LABORATORY SAFETY

2.1 Safe laboratory practices must be followed at all times, and should be outlined in a laboratory safety manual that is kept in the laboratory at all times.

2.2 Eye protection *(i.e.*, laboratory safety glasses or approved prescription glasses) must be worn when pouring solvents or preservatives (*e.g.*, formalin) handling chemicals, or when performing any procedures that require use of a fume hood. Safety glasses are available in the laboratory.

2.3 Repeated exposure to formaldehyde can increase sensitivity to this potentially harmful chemical. Exposure should be minimized by handling formaldehyde and preserved samples with gloves, conducting all work involving formaldehyde under a fume hood, using portable ventilation devices over microscopes when sorting preserved samples, and storing samples in a well-ventilated room away from areas in which personnel are working.

2.4 Waste Disposal

2.4.1 No chemicals are to be disposed through laboratory drains leading to a municipal or private sewer or septic system. Chemicals must be disposed in appropriate containers available in the laboratory.

2.4.2 Diluted formaldehyde may, in certain cases, be disposed through laboratory sewer lines. Permitted discharge volumes and concentrations will depend upon state and local regulations.

2.5 Work areas are to be kept clean and neat at all times.

2.6 Any conditions that hinder the accomplishment of work or represent a possible safety hazard should be brought to the attention of the laboratory supervisor immediately.

3. TRAINING

3.1 A program the size of EMAP-Estuaries involves many field and laboratory technicians. Standard procedures must be followed to ensure that data produced by multiple laboratories and different personnel are comparable.

3.2 All laboratory personnel will have a minimum basic level of training. Instruction will include evaluation and proficiency testing to insure the mastery of skills.

3.3 Training will be provided by experienced personnel in established laboratories. New employees will learn laboratory techniques using practice or "dummy" samples and in no case will handle, without supervision, real EMAP samples without prior demonstration of acceptable proficiency.

3.4 The overall proficiency of laboratory personnel will be continuously evaluated using the various quality assurance and quality control procedures outlined in the methodologies given below. The status of personnel demonstrating substandard performance will be reevaluated, and, if necessary, personnel will be retrained or removed from the laboratory to maintain the consistent, high quality production of data.

4. SAMPLE STORAGE AND TREATMENT

4.1 Sample processing starts with the reception of samples from field crews. Samples are bar coded with a unique sample number. Upon receipt, a bar code reader will be used to log samples into computer files; sample identification numbers will be checked against a master list provided by the EMAP-Estuaries Province Field Operations Center. In addition, the data files containing the records of all samples received by the laboratory will be transferred to the Province Field Operations Center at regular intervals for the purpose of sample tracking. Discrepancies, missing, or damaged samples will be reported to the Province Manager and/or Field Coordinator immediately.

4.2 Within the laboratory, all samples will be carefully tracked by sample number using a sample log. It will be the responsibility of each laboratory to keep accurate and timely records of the location and status of all samples in their custody.

4.3 Stored samples must be easily retrieved and protected from environmental extremes. Samples cannot be allowed to freeze and should be stored above 5°C to prevent the formation of paraformaldehyde (Jones 1976). Temperatures greater than about 30°C should be avoided to retard evaporative losses. Stored and archived samples should be checked once every three months to avoid excessive evaporative losses due to loosely fitting or cracked container lids or inadequately sealed jars. Exposure to direct sunlight should be minimized since long-term exposure can degrade the vital rose bengal stain.

4.4 Previously fixed samples that are being processed in the laboratory should be represerved in a 70 percent (volume/volume) ethanol solution. Samples should not remain in water without preservative for more than 24 hours since microbial degradation may occur. Degradation may impact subsequent biomass measurements.

4.5 Differential Treatment of Samples Due to Salinity:

4.5.1 Sample treatment is dependent upon the salinity of the habitat from which samples were taken. Benthic samples from tidal fresh and oligohaline salinity zones (0 to 5 ‰) will be treated differently than samples from mesohaline and polyhaline salinity zones (> 5 ‰).

4.5.2 Oligochaetes and chironomids from tidal fresh and oligohaline salinity zones will be identified to species, where possible, whereas individuals of these groups from higher salinities will not be further differentiated.

4.5.3 The rationale for this inconsistent treatment of samples is that the additional taxonomic effort necessary to identify oligochaetes and chironomids to the species level produces important and useful information for the interpretation of benthic communities in tidal fresh and oligohaline regions, but produces information of marginal value in mesohaline and polyhaline environments. This is largely due to the greater diversity of these two groups in lower salinity environments.

4.5.4 Salinity categories (tidal fresh and oligohaline vs. mesohaline and polyhaline) will be determined using bottom salinity values collected at the time benthic grabs were taken. Laboratory processing of

benthic samples will not proceed without salinity data for the sampling station or area from which benthic samples were collected.

4.5.5 In cases where the salinity for a sample is unknown and the species assemblage is insufficient to place the sample in a specific salinity class, the sample will be treated as a tidal fresh/oligohaline sample. This will insure that no potentially useful information will be lost due to the treatment of samples.

4.5.6 Sample replicates from the same station will be treated similarly. All replicates will be processed as tidal fresh/oligohaline or as mesohaline/polyhaline/samples.

5. SAMPLE SIEVING, SORTING, SPECIES IDENTIFICATION, AND ENUMERATION

5.1 The objective of sorting benthic samples is to completely remove from sample debris all fauna of interest that were alive at the time of collection. Sample debris includes primarily sediment, but also detritus and the remnants (death assemblage) of the hard parts of various benthic organisms (for example, the shells of bivalve mollusks or the exoskeletons of crustaceans).

5.2 Benthic Fauna of Interest

5.2.1 The fauna of interest to EMAP-Estuaries are benthic macrofauna operationally defined here as those metazoan organisms retained by a 0.5 mm (500 µm) mesh sieve. All fauna retained on the 0.5 mm sieve will be identified, enumerated, and included as macrofauna, except those meiofaunal and pelagic groups specified in paragraphs below. No upper size limit for macrofauna will be used by the program.

5.2.2 Faunal groups, typically termed meiofauna, for which the majority of individuals are too small to be retained on a 500 μm sieve will not be identified, enumerated, or processed for biomass measurements. These groups include the following:

Turbellarian flatworms
Kinorhynchs
Nematodes
Harpacticoid copepods
Cyclopoid copepods
Ostracods
Halacarids.

5.2.3 Faunal groups that are clearly pelagic and not benthic will not be identified, enumerated, or measured for biomass. Examples of pelagic groups include the following:

Cladocerans
Calanoid copepods.

5.2.4 Eggs or egg cases will not be identified, enumerated, or measured for biomass. Only individuals that have fully separated from their eggs will be counted.

5.2.5 Flying insects that may have been collected in samples, will not be identified, enumerated, or measured for biomass.

5.3 Sieving:

5.3.1 Benthic samples were previously sieved through 0.5 mm mesh sieves in the field to remove most sediment particles less than 0.5 mm (see Field Operations and Methods Manual). All samples will be resieved in the laboratory using 0.5 mm mesh sieves to ensure each sample is consistently and completed processed. Samples will be sieved in the following step-wise manner:

5.3.2 Throughout laboratory processing, all samples will be tracked by the sample number given prior to field sampling.

5.3.3 Sieves will be cleaned and backwashed thoroughly before processing each sample.

5.3.4 Under a fume hood, the sample will be poured through the sieve. The filtrate has a 3.7% concentration of formaldehyde and will be saved in a properly labeled container for later disposal or reuse.

5.3.5 Using tap water, any portion of the sample remaining in the jar will be rinsed into the sieve.

5.3.6 The sieve containing the sample will be placed in a wash basin. The basin will be filled with water and agitated to wash fine material through the sieve. This procedure minimizes mechanical damage to fragile fauna. A gentle spray of water may also be used to wash material through the sieve, but direct, heavy jets of water should not be used.

5.3.7 Using a gentle spray of water, material will be transferred from the sieve into a labeled container in preparation for sorting.

5.3.8 Sieves will be examined after rinsing to ensure that all organisms have been removed and to minimize cross contamination with the next sample.

5.4 Sorting:

5.4.1 All macrofauna that were alive at the time of collection must be separated and removed from organic debris and sediment particles remaining after sieving. All organisms, including significant body fragments, must be saved to obtain correct estimates of density and to make accurate biomass determinations. Unidentifiable material must be saved until positively identified.

5.4.2 Sorting commences by pouring sieved material into white enamel or plastic trays for initial removal of larger organisms. Finer material will be transferred to a grided petri dish for sorting using a stereomicroscope. Samples must be evenly distributed over the tray or petri dish and the water level must

be low enough to prevent sloshing back and forth as the tray or dish is moved.

5.4.3 Organisms will be removed as the tray or petri dish is systematically searched. Organisms will be divided into major taxa, typically polychaetes, oligochaetes, bivalves, gastropods, crustaceans, etc.

5.4.4 Laboratory personnel completing the initial sorting normally do not complete identifications to the species level. Species identifications are completed by taxonomists who have more training and experience than sorters.

5.4.5 Organisms may be identified by taxonomists to species level immediately or may be saved for later identification. Fauna will be sorted into major taxonomic groups, placed into small screw-top vials, and preserved in ethanol. Vials will contain labels indicating the sample number and taxon. Vials from the sample will be bound together with rubber bands.

5.4.6 The number of vials for each sample will be recorded in the sample log (sample tracking sheet) along with the initials of the sorter completing this part of the laboratory processing.

5.4.7 Sample debris that remains after sorting will be transferred from the petri dish to a jar, preserved in a 70% (volume/volume) ethanol solution, labeled, and for each technician, saved in batches of 10. A log will be kept of all archived samples. Ten percent of each batch will be resorted as a quality control check on each sorter's efficiency.

5.4.8 Sample debris in each sample batch may be discarded after quality control procedures have been completed and approved.

5.4.9 Sorting efficiencies meeting or exceeding the measurement quality objective of 90% will be assured by several measures: only qualified technicians trained to process EMAP-Estuaries samples will sort samples; the sample sorting protocol will be documented and uniformly applied to all samples; and all sorting will be closely and continuously monitored by supervisory staff. In combination, the application of training, supervision, and controlled laboratory procedures will ensure that all samples will be processed correctly, and that resulting data will not be invalidated by contamination, loss of vials, or incomplete removal of organisms from the sample.

5.5 Species Identification and Enumeration:

5.5.1 The identification of biological specimens to the species level requires specialized taxonomic training, experience, and a familiarity with current taxonomic literature. The validity of taxonomic identifications affects the quality of subsequent population and community analyses, as well as the comparability of the research to other studies; therefore, only qualified and experienced technicians will perform species identifications. Typically, no one person can completely master the taxonomic complexities of all benthic macrofaunal groups. For example, in any laboratory, one technician might develop an expertise in identifying polychaetes; another may be better at identifying gastropods. All technicians will be trained to recognize commonly occurring species. Rarer species will require the taxonomic expertise of specialists

within the laboratory, and may, at times, require the assistance of expert taxonomists in other laboratories. A list of taxonomic experts will be maintained by EMAP-Estuaries.

5.5.2 The objective of species identification and enumeration is to accurately identify all organisms found in a sample to the lowest possible taxonomic category consistent with study objectives and to accurately count the number of organisms in each taxon. For EMAP-Estuaries, specimens will be identified to the species level whenever possible. The usefulness of such efforts in environmental monitoring programs is well established (Grassle and Grassle, 1984).

5.5.3 Due to various taxonomic difficulties, certain groups expected to be encountered by EMAP-Estuaries will not be identified to species. These groups and the associated level of expected taxonomic classification include the following:

Taxonomic Group	Degree of Expected Classification
Phylum Nemertinea (Proboscis worms)	Phylum
Phylum Sipuncula	Phylum
Class Oligochaeta	Class*
Class Hirudinea	Class
Class Anthozoa	Class
Family Chironomidae	Family*

*In marine, polyhaline, and mesohaline regions only. Identifications will be made to species level, if possible, in oligohaline and tidal fresh regions.

5.5.4 The number of individuals counted for each taxon must reflect the number of organisms alive at the time of sampling; therefore, when organism fragments are recovered, counts will be based upon only the number of heads found. Posterior body fragments will not be counted, but must be retained with the appropriate taxonomic group for biomass determinations. If only posterior fragments are present (no heads), they will be counted as one individual unless a greater number of individuals can be positively identified. In that case, the greater number of individuals will be recorded.

5.5.5 All identifications will be performed under a high quality dissecting microscope with sufficient magnification for clear resolution of morphological details; a microscope with 5 to 50x power is usually sufficient. On occasion, a compound microscope capable of higher magnification may be required and should be available for use.

5.5.6 Wherever possible, different taxonomists will identify specimens in replicate samples taken at the same station and time. Alternatively, the same taxonomist will process replicates on different days.

5.5.7 Sample processing for identification and enumeration commences by retrieving sample vials for a particular sample from the previous sorting procedure. At that time, a species identification data sheet is started. The sample number on the vials will be checked with that recorded in the sample log; the number of vials should match the number noted in the log.

5.5.8 Specimens from each vial will be rinsed into separate petri dishes. Care must be taken to remove all specimens from the vial, both the vial and cap will be checked for remaining specimens.

5.5.9 Specimens will be identified, counted, and removed from the petri dish one at a time.

5.5.10 Identified organisms will be grouped by the categories to be used in biomass determinations (Subsection 6.6), placed in vials, and preserved in 70% ethanol. Vials will be labeled with sample number and biomass group. The appropriate biomass category will be marked with a check on the biomass data sheet which accompanies each abundance data sheet.

5.5.11 Petri dishes will be thoroughly inspected for missed specimens and then will be rinsed to minimize cross contamination between vials and samples.

5.5.12 Identified species will be checked for suspicious mismatches as part of the identification process. An example of a suspicious mismatch is the identification of a freshwater benthic species in a sample from a polyhaline salinity region.

5.5.13 Specimens that are difficult to identify will be set aside in vials and preserved in ethanol for future study. Some specimens will require the expertise of more experienced technicians in the same laboratory. Other specimens may require further laboratory processing (for example, oligochaetes and chironomids will need to be mounted on microscope slides) before species determination can be made. Still other specimens may need to be sent to other laboratories to complete species identifications. The location of all specimens for a particular sample will be tracked using the species identification data sheet and the laboratory sample log.

5.5.14 Treatment of Oligochaetes and Chironomids

5.5.14.1 In general, all specimens will be identified and enumerated from visual inspection using a stereomicroscope. However, certain taxonomic groups will require special handling to optimize both species identification and biomass determination. Those two groups are the Class Oligochaeta and the Family Chironomidae. Specimens of both groups in samples from tidal fresh and oligohaline regions will need to be inspected under high magnification using a compound microscope to properly complete species identifications. Sample processing of oligochaetes and chironomids will proceed in the following manner for each group.

5.5.14.2 For each sample and for each group (oligochaetes and chironomids), if less than 20 individuals are found in a sample, all individuals will be permanently mounted on a microscope slide and identified to the species level when possible. No biomass determination will be made for these samples. Since most oligochaetes and chironomides are relatively small, this will not cause underestimation of a significant amount of biomass.

5.5.14.3 If the number of specimens is between 20 and 400, then approximately 50% of the specimens will be mounted and the remaining portion will be measured for biomass. Samples will be split in the following manner:

5.5.14.3.1 Specimens will be distributed in a grided tray as evenly as possible. Grids will be selected at random until exactly half of the total number of grids are selected. The specimens in these grids will be mounted and identified. Due to the random distribution of fauna, half of the sample grids will not necessarily contain 50% of the specimens.

5.5.14.3.2 Specimens in the remaining grids will be enumerated, combined, and processed for biomass.

5.5.14.4 If the number of specimens is greater than 400, samples will be treated in the following manner:

5.5.14.4.1 Samples will be split as in 5.5.14.3.1. Instead of using half of the total number of grids to select specimens to be identified, grids will be randomly selected until at least 200 specimens are mounted. Any remaining specimens in the last selected grid will also be identified. The total number of identified specimens in these samples will usually be greater than 200.

5.5.14.4.2 Specimens in the remaining grids will be enumerated, combined, and processed for biomass.

5.5.15 Count of the number of species in each sample.

5.5.15.1 Each species will be included in the count of the total number of species in each sample.

5.5.15.2 Specimens that can be identified only to genus, family, or order will also be included in the total number of species in each sample (*e.g.*, specimens identified to be within family Spionidae will be counted as one species). If a specimen identified to genus, family, or order can be identified as one of several species already identified in the sample, that specimen will not be counted as an additional species. This procedure eliminates double counting of certain species. For example, a specimen is identified as being *Tellinidae*, but the taxonomist believes it to be either *Macoma balthica* or *Macoma mitchelli*, both of which are present in the sample. The specimen would be recorded as *Tellinidae* and would not be included in the species count for that sample.

5.5.15.3 Identified specimens that will not be included in species counts will be so marked by recording a '1' in the appropriate column on the abundance data sheets.

5.5.15.4 Fragments of organisms generally will not contribute to the count of number of species per sample; however, if a fragment contributes to abundance by meeting the criteria outlined in Subsection 5.5.4, then fragments will also contribute to the count of the number of species per sample.

5.5.16 Additional quality control procedures for species identification and enumeration, including maintenance of a laboratory taxonomic reference collection, are given in Subsection 7.

6. MACROFAUNAL BIOMASS DETERMINATION

6.1 Biomass is an additional measure of the overall health, status, and history of benthic communities. This section describes the methods to be used to measure benthic biomass. Biomass will be reported as formaldehyde dry weight since samples were fixed and initially preserved in formaldehyde. To ensure that measurements are standardized and that comparisons may be made between samples and between taxonomic groups, two factors must be considered. First, biomass measurements must be made only after samples have been preserved for a certain minimum time, and, second, soft bodied organisms and those having significant inorganic body parts must be treated separately.

6.2 Biological samples lose weight when preserved in formalin due to the precipitation of proteins (Jones 1976). Weight loss is variable and is related to organism composition and duration of preservation. In general, weight loss decreases exponentially and is negligible after two months (Howmiller 1972; Schram *et al.* 1981; Mills *et al*. 1982). In order to standardize measurements, all samples will be preserved in a 10% formalin solution for at least one month before biomass measurements are made. Subsequent transfer to and represervation in ethanol is not thought to impact biomass measurements.

6.3 Biomass measurements cannot be made until all specimens in a sample have been properly identified and all quality control procedures have been implemented. Measuring biomass is a destructive process after which no further taxonomic identifications or checks are possible.

6.4 Soft-bodied organisms (*e.g*., amphipods and polychaetes) may be dried and weighed immediately after identification. Hard-bodied organisms (*e.g.*, bivalves, gastropods, and echinoderms) will need to be acidified prior to drying and weighing. Acidification removes the calcium carbonate present leaving behind organic carbon. Acidification procedures are given in Subsection 6.10.

6.5 An analytical balance with an accuracy of 0.1 mg will be used to measure biomass.

6.6 Biomass will be individually determined for the most dominant macrofaunal species or group of species in each Province. Dominant species or groups of species are selected prior to laboratory processing based upon a review of published studies. Remaining species are grouped into categories having ecological or taxonomic relevance. Biomass categories selected for the Virginian Province are as follows:

Amphipod Biomass Groups:

Ampelisca spp.

Corophium spp.

Gammaridae

Haustoriidae

Leptocheirus spp.

Monoculodes spp.

Unciola spp.

Other and unidentifiable amphipods

Arthropod Biomass Groups:

Cyathura spp.

Other and unidentifiable isopods

Decapods

Chironomids

Polychaete Biomass Groups:

Glycera spp.

Heteromastus filiformis

Leitoscoloplos spp.

Maldanidae

Marenzelleria viridis

Mediomastus ambiseta

Neanthes succinea

Nephtys spp.

Paraprionospio pinnata

Polydora spp.

Streblospio benedicti

Other polychaetes - sub-surface deposit

feeders

Other polychaetes - surface deposit/suspension feeders

Other polychaetes - carnivores/omnivores

Other and unidentifiable polychaetes and polychaete fragments

Oligochaete Biomass Group

All oligochaetes and oligochaete fragments

Bivalve Biomass Groups

Corbicula fluminea

Ensis directus

Gemma Gemma

Mercenaria mercenaria

Mulinia lateralis

Nucula spp.

Rangia cuneata

Tellinidae

Yoldia limatula

Other bivalves - deposit feeders

Other bivalves - suspension feeders

Other and unidentifiable bivalves and bivalve fragments

Gastropods Biomass Groups:

Acteocina canaliculata

Hydrobia spp.

Other and unidentifiable gastropods

Miscellaneous Species and Groups:

Echinodermata

Hemichordata

Miscellaneous Species and Groups:

Phoronis spp.

Nemertinea

Other species and unidentifiable macrofauna

6.7 The measurement of biomass for each of the groups above will commence with the collection of the species identification data sheet and taxon storage vials for an individual sample. Biomass data sheets will accompany abundance sheets and will note how many and which biomass vials should be present. Any discrepancies between data sheets and vials will be corrected at this time.

6.8 In the steps that follow, the treatment for soft-bodied and hard-bodied macrofaunal organisms differs. The treatment for soft-bodied organisms will be given first and will be followed by the treatment for hard-bodied organisms.

6.9. Soft-Bodied Macrofauna:

6.9.1 Soft bodied organisms will be placed in preweighed, numbered weighing pans. An appropriately sized pan will be selected for each taxonomic category according to the amount of material to be processed. The pan number will be recorded on the biomass data sheet along with the taxonomic group to be measured.

6.9.2 Care will be taken to check that all organisms are rinsed from the vials into the weighing pan.

6.10 Hard-Bodied Macrofauna:

6.10.1 Hard bodied organisms will be placed in preweighed, numbered, porcelain crucibles. A crucible of appropriate size will be selected for each taxonomic category according to the amount of material to be processed. The crucible number will be recorded on the biomass data sheet along with the taxonomic group to be measured.

6.10.2 Care will be taken to check that all organisms are rinsed from the vials into the crucible.

6.10.3 Large bivalves (length > 2 cm) will be shucked instead of being acidified; acidification of large shells is time consuming and uses an excessive amount of acid.

6.10.4 Crucibles will be acidified in a fume hood using 10% HCl. Acidification will continue until all visible traces of shell material are removed. Additional acid may be added as needed to bring about the complete dissolution of shell material.

6.10.5 When no traces of shell material remain, the acid will be removed with a pipette and the remaining material will be rinsed with distilled water.

6.11 In subsequent steps, soft-bodied and hard-bodied macrofauna will be similarly treated.

6.12 Weighing pans and crucibles will be placed in carrying trays and dried in an oven at 60°C.

6.13 Pans and crucibles to be weighed will be grouped in batches. The batch number will be recorded on biomass data sheets. Each batch will contain weighing blanks. Blanks are pans and crucibles which have been treated as biomass samples but to which no fauna have been added. Blanks will be used to

determine the overall accuracy of the weighing procedure and will help detect errors due to the contamination of biomass samples. Approximately 5 to 10% of the number of pans and crucibles in a batch will be blanks.

6.14 Prior to weighing pans and crucibles, the balance will be zeroed and a standard weight will be used to test its calibration. Each standard is individually numbered and this number will be noted in the biomass log book. Subsequent weighings will use the same standard weight.

6.15 Dried samples awaiting measurement will be stored in a desiccator to avoid absorbing moisture from the atmosphere. Desiccator storage also allows samples to cool to room temperature prior to weighing.

6.16 Typically, 24 to 48 hours at 60 °C is sufficient for the dry weight of benthic samples to stabilize, however, some samples may take longer to dry. As a check, all samples will be weighed after 24 hours, the weight will be recorded on the biomass data sheets, and the samples will be returned to the drying oven. Dry weight will be measured again after 24 hours. If the second sample weight differs from the first by more than 5%, then the sample will be returned to the drying oven for an additional 24 hours. This cycle will be repeated until a stable dry weight measurement is obtained. All weights will be recorded on the biomass data sheet.

6.17 Approximately 10% of all pans and crucibles will be reweighed by a second technician as a quality control check of biomass measurements.

6.18 Biomass data sheets will contain a record of all weights; however, only the tare weight of weighing pans and crucibles and the final weight of the sample (sample weight + crucible or pan weight) will be entered into the data base. Net (sample) weights will be calculated within the data base.

6.19 Weighing blanks should vary by no more than 0.3 mg. If greater variations are found, the balance and the weighing procedures used by the technician should be checked, and, as necessary, the balance will be repaired or the technician will be retrained.

7. QUALITY ASSURANCE AND QUALITY CONTROL

7.1 Various quality control (QC) procedures will be implemented to ensure consistent production of high quality data. In addition to the QC procedures included in this chapter, the following procedures will be periodically conducted as part of data quality control.

7.2 Sorting:

7.2.1 A minimum of 10% of all samples sorted by each technician will be resorted to monitor technician performance and provide feedback necessary to maintain acceptable standards. Resorts will be conducted on a regular basis on batches of 10 samples, and all results will be documented and recorded in the QA/QC logbook for the laboratory.

7.2.2 The QC resort procedure is designed to provide effective and continuous monitoring of sorting efficiency. For EMAP-Estuaries, the minimum acceptable sorting efficiency is 90%. Based upon the experience of other programs using similar methods (Holland *et al.* 1988), however, sorting efficiencies are expected to be greater than 95%.

7.2.3 Samples sorted by a particular technician will be randomly selected for resorting from a sample batch.

7.2.4 The archived sample residues will be retrieved and the sample number will be recorded in the QC log book.

7.2.5 The residue will be resorted using the sorting procedures given in Subsection 5.4.

7.2.6 Sorting efficiency (%) will be calculated using the following formula:

$$\frac{\#\ organisms\ originally\ sorted}{\#\ organisms\ originally\ sorted\ +\ additional\ \#\ found\ in\ resort}\ x\ 100$$

7.2.7 The results of sample resorts may require that certain actions be taken for specific technicians. If sorting efficiency is greater than 95%, no action will be required. If sorting efficiency is 90 to 95%, the technician will be retrained and problem areas identified. Laboratory personnel and supervisors must be particularly sensitive to systematic errors (*i.e.*, consistent failure to represent specific taxonomic groups) that may suggest the need for further training. Resort efficiencies below 90% will require resorting all samples in that batch and continuous monitoring of that technician to improve efficiency.

7.2.8 If sorting efficiency is less than 90%, organisms found in the resort will be added to the original data sheet and placed in the appropriate biomass group vial. If sorting efficiency is 90% or greater, the results will be recorded in the QC log book; however, the animals should be kept separate from the original sample and should not be used for biomass determinations.

7.2.9 If a sample batch fails to meet the 90% efficiency sorting criteria, all samples within the batch will be resorted. An additional sample from the batch will be randomly selected and used to check the sorting efficiency of the resorted batch.

7.2.10 After resorting, and if quality control criteria are met, sample residues may be discarded.

7.2.11 Resort results will be summarized for each technician on a QC resort summary sheet.

7.3 Species Identification and Enumeration:

7.3.1 Only senior taxonomists are qualified to complete identification quality control checks. A minimum of 10% of all samples processed by each taxonomic technician will be checked to verify the accuracy of species identifications and enumerations. This control check establishes the level of accuracy with which

identification and counts are performed and offers feedback to taxonomists in the laboratory to maintain a high standard of accuracy. Samples will never be rechecked by the technician who originally processed the sample.

7.3.2 Approximately 10% of each sample batch will be checked. A sample batch consists of 10 samples and ideally is made of samples from a similar habitat type (all oligohaline stations, for example). Rechecks will be performed in a timely manner so that subsequent processing steps (*e.g.,* biomass determinations) and data entry may proceed.

7.3.3 The vials containing specimens from the randomly selected sample will be retrieved along with the original species identification sheet and information will be recorded in the QC log book.

7.3.4 The specimens in each vial will be reidentified and enumerated using the procedures given in Subsection 5.5 of this chapter.

7.3.5 As each taxon is identified and counted, results will be compared to the original data sheet. Discrepancies will be double-checked to verify that the final results are correct.

7.3.6 Following reidentification, specimens will be returned to the original vials and set aside for biomass determination.

7.3.7 When the entire sample has been reidentified, the total number of errors will be computed. The total number of errors will be based upon the number of misidentifications and miscounts. Numerically, accuracy will be represented in the following manner:

$$\frac{\textit{Total \# of organisms in QC recount} - \textit{total no. of errors} *}{\textit{Total \# of organisms in QC recount}} * 100$$

* Three types of errors are included in the total number of errors:

1) Counting errors (for example, counting 11 *Gemma gemma* as 10)

2) Identification errors (for example, identifying a *Nucula annulata* specimen as *Nucula proxima*, where both are present)

3) Unrecorded taxa errors (for example, not identifying *Phoronis* spp. when it is present)

7.3.8 For EMAP-Estuaries benthic samples, the minimum acceptable taxonomic efficiency will be 90%. If taxonomic efficiency is greater than 95%, no action will be required. If taxonomic efficiency is 90 to 95%, the taxonomist will be consulted and problem areas will be identified. Taxonomists and laboratory supervisors must be particularly sensitive to systematic errors (*i.e.*, repeated errors for specific taxonomic groups) that may suggest the need for further training. Taxonomic efficiencies below 90% will require reidentifying and enumerating all samples in that sample batch and additional monitoring of the taxonomist to improve efficiency.

7.3.9 Any species identification changes resulting from quality assurance procedures will be recorded on the original data sheet; however, the numerical count for each taxonomic group will not be corrected unless the overall accuracy for the sample is below 90%.

7.3.10 Treatment of the results of quality control audits are illustrated in the following examples.

7.3.10.1 Example 1: Ten *Mulinia lateralis* individuals were recounted as eleven. The sample had a greater than 90% overall efficiency, therefore, the original count of ten *Mulinia* would be recorded.

7.3.10.2 Example 2: One individual of the species *Prionospio steenstrupi* was misidentified as *Streblospio benedicti*. On the final data sheet, one *Prionospio steenstrupi* and no *Streblospio benedicti* would be recorded.

7.3.10.3 Example 3: Ten *Nucula annulata* and no *Nucula proxima* were originally recorded. During the QA/QC check, one *N. annulata* was found to be *N. proxima.* Providing the overall efficiency was greater than 90%, nine *N. annulata* and one *N. proxima* would be recorded on the final data sheet.

7.3.10.4 Example 4: Five *Nucula annulata* and ten *Mulinia lateralis* were originally recorded. During the QA/QC check, one *M. lateralis* was found to be a *N. annulata*. Providing the overall efficiency was greater than 90%, six *N. annulata* and nine *M. lateralis* would be recorded on the final data sheet.

7.3.10.5 Example 5: One Onuphidae spp. (juvenile) was recorded on original data sheet. During the QA/QC check, this individual was not found. On the final data sheet, one Onuphidae spp. (juvenile) would be recorded.

7.3.10.6 Example 6: Terebellidae spp. (juvenile) was found in the annelid fragment category during the QA/QC check. No Terebellidae were previously recorded on the data sheet. On the final data sheet, one Terebellidae spp. would be recorded.

7.3.11 The results from all QC rechecks of species identification and enumeration will be recorded in the QC log book that will become a part of the documentation for EMAP-Estuaries.

7.3.12 All corrections to data sheets will be initialed and dated by the person making the changes.

7.4 Taxonomic Reference Collection:

7.4.1 Taxonomic identifications should be consistent within a given laboratory and with the identifications of other regional laboratories. Consistent identifications are achieved by implementing the procedures described above and by maintaining informal interaction among the taxonomists working on each major group.

7.4.2 A voucher specimen collection should be established by each laboratory processing EMAP samples. This collection should consist of representative specimens of each species identified in samples from an

individual province. For some species, it may be appropriate to include in the voucher specimen collection individuals sampled from different geographic locations within the Province.

7.4.3 New species added to a laboratory's voucher specimen collection should be sent to recognized experts for verification of the laboratory's taxonomic identifications. The verified specimens should then be placed in a permanent taxonomic reference collection. The reference collection should be used to train new taxonomists. Participation of the laboratory staff in a regional taxonomic standardization program (if available) is recommended, to ensure regional consistency and accuracy of identification.

7.4.4 All specimens in the reference collection should be preserved in 70% ethanol in labeled vials that are segregated by species and sample. More than one specimen may be in each vial. The labels placed in these vials should be made of waterproof, 100-percent rag paper and filled out using a pencil. Paper with less than a 100-percent rag content or that is not waterproofed will disintegrate in the 70-percent alcohol mixture. It is important to complete these labels because future workers may not be familiar with details of the work in progress.

7.4.5 To reduce evaporation of alcohol, the lids of vials and jars can be sealed with plastic tape wrapped in a clockwise direction. The species (and other taxonomic designation) should be written clearly on the outside and on an internal label. Reference specimens should be archived alphabetically within major taxonomic groups.

7.4.6 Reference collections are invaluable and should be retained at the location where the identifications were performed. In no instance should this collection be destroyed. A single person should be identified as curator of the reference collection and should be responsible for its integrity. Its upkeep will require periodic checking to ensure that alcohol levels are adequate. When refilling the jars, it is advisable to use full-strength alcohol (*i.e.*, 95 percent), because alcohol tends to evaporate more rapidly than water in a 70-percent solution.

7.4.7 The laboratory will maintain a log pertaining to the taxonomic reference collection. This log will contain the species name, the name and affiliation of the person who originated the reference sample, the location of the reference sample, the status of the sample if it has been loaned to outside experts, and information about the confirmation of identification by outside experts. The log may also contain references to pertinent literature describing the species in the reference sample.

7.5 Biomass Measurements:

7.5.1 A minimum of 10% of all pans and crucibles in each batch will be reweighed to monitor technician performance and to provide the feedback necessary to maintain acceptable standards. Reweighings will be conducted on a regular basis on sample batches, and all results will be documented and recorded in the laboratory QA/QC log book for the laboratory.

7.5.2 Samples to be reweighed will be randomly selected from a sample batch.

7.5.3 Selected samples will be reweighed and the results compared against the final weight recorded on the biomass data sheet.

7.5.4 Weighing efficiency will be calculated using the following formula:

$$\frac{|\textit{Original Final Weight} - \textit{Reweighted Final Weight}|}{\textit{Reweighed Final Weight}} \times 100$$

7.5.5 If weighing efficiency is 95% or greater, the sample has met the acceptable quality control criteria and no further action is necessary. If the weighing efficiency is between 90% and 95%, the sample has met acceptable criteria, but the technician who completed the original weighing will be consulted and proper measurement practices reviewed. If the weighing efficiency is less than 90%, the sample has failed the quality control criteria and all samples within the sample batch must be reweighed. Additionally, the performance of the original technician will be reviewed and the technician will be retrained.

7.5.6 Correction to the original data sheet will be made only in those cases where weighing efficiency is less than 90%.

7.5.7 The results of all QC reweighings will be recorded in the QC log book that will become a part of the documentation for EMAP-Estuaries.

8. DATA MANAGEMENT

8.1 All data generated in the laboratory will be recorded directly onto standardized data forms. Data forms will be designed so that all necessary information will be recorded clearly and unambiguously. All data will be recorded in ink. Data forms will be linked to specific samples using the bar coded sample numbers that were assigned by the Province Field Operations Center prior to field sampling. Completed data forms will be kept in bound notebooks arranged by type. Completed data forms will be made available to the Province Field Operations Center upon demand. Laboratories will contact the EMAP-Estuaries Technical Director prior to the disposal of any data sheet, QA/QC forms, or laboratory notebooks pertaining to the generation of EMAP data. The length of time these materials must be archived by contract laboratories may be determined by contract.

9. DATA FORMS

9.1 This section lists data forms that will be used for the identification, enumeration, and determination of biomass for benthic macrofaunal samples collected by EMAP-Estuaries in the Virginian Biogeographic Province (Cape Cod, MA to Cape Henry, VA). Sample data forms are included in Appendix A. Data forms are presented in the following order:

- Abundance data sheet for tidal fresh and oligohaline regions (1 page)

- Abundance data sheet for mesohaline regions (1 page)
- Abundance data sheets for southern polyhaline regions (2 pages)
- Abundance data sheets for northern polyhaline region (2 pages)
- Biomass data sheets for tidal fresh and oligohaline salinity regions (2 pages)
- Biomass data sheets for mesohaline and polyhaline salinity regions (2 pages)
- Laboratory Sample Tracking form (Benthos log sheet 1 page)
- QC Sample Batch Listing form (1 page)
- QC Sample Resort sheet (1 page)
- QC Sample Reidentification sheet (1 page)
- Biomass log sheet (1 page)
- QC Biomass Reweighing and Blank sheet (1 page)

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APPENDIX A

DATA FORMS

Page ___ of ___

NEAR-COASTAL EMAP 1993
Species Abundance Data Sheet
Tidal Freshwater / Oligohaline

EXAMPLE

SAMPLE: EXAMPLE Priority 1

LAB: Versar ESM Operations | OLIGO. SPLIT: / CHIRON. SPLIT: /

SORT DATE: (m d y) SORTED BY: | ID DATE: (m d y) ID BY:

MOUNT DATE: M. ID BY: | SYSTEM: Chesapeake Bay Maryland

BM Gp	Species	Code	Num	*
46	Turbellaria	TURBELLA		
29	Corbicula fluminea	CORBFLUM		
28	Macoma mitchelli	MACOMITC		
38	Musculium spp.	MUSCULIU		
38	Musculium transversum	MUSCTRAN		
38	Pisidium spp.	PISIDIUM		
34	Rangia cuneata	RANGCUNE		
27	Aulodrilus limnobius	AULOLIMN		
27	Aulodrilus pigueti	AULOPIGU		
27	Dero digitata	DERODIGI		
27	Dero spp.	DERO		
27	Ilyodrilus templetoni	ILYOTEMP		
27	Limnodrilus cervix	LIMNCERV		
27	Limnodrilus hoffmeisteri	LIMNHOFF		
27	Limnodrilus udekemianus	LIMNUDEK		
27	Quistadrilus multisetosus	QUISMULT		
27	Tubificidae w/o cap	TUBIFIWI		
27	Tubificidae wth cap	TUBIFIWO		
27	Tubificoides heterochaetus	TUBIHETE		
25	Hobsonia florida	HOBSFLOR		
18	Marenzelleria viridis	MAREVIRI		
2	Corophium lacustre	COROLACU		
3	Gammarus daiberi	GAMMDAIB		
3	Gammarus fasciatus	GAMMFASC		
3	Gammarus spp.	GAMMARUS		

BM Gp	Species	Code	Num Dir	Num Mnt	*
5	Leptocheirus plumulosus	LEPTPLUM			
6	Monoculodes sp. 1 Watling	MONOSPE1			
12	Chironomidae	CHRNMDAE			
12	Chironomus spp.	CHIRONOM			
12	Coelotanypus spp.	COELOTAN			
12	Cryptochironomus fulvus	CRYPFULV			
12	Cryptochironomus spp.	CRYPTOCH			
12	Polypedilum convictum	POLYCONV			
12	Polypedilum spp.	POLYPEDI			
12	Procladius spp.	PROCLADI			
12	Procladius sublettei	PROCSUBL			
12	Tanytarsus spp.	TANYTARS			
46	Chaoborus punctipennis	CHAOPUNC			
10	Chiridotea almyra	CHIRALMY			
9	Cyathura polita	CYATPOLI			

Temporary Record - Do not enter into data base

	Oligochaetes - Total Split	XXXXXXXX		
	Chironomids - Total Split	XXXXXXXX		

*: Write '1' in this column if taxon is NOT to be considered when counting species in sample

Num=# of Individuals

Number of Biomass Vials

Page ___ of ___

NEAR-COASTAL EMAP 1993
Species Abundance Data Sheet
Mesohaline

EXAMPLE

SAMPLE: EXAMPLE Priority 1

LAB: Cove Corporation

LAB SAMPLE #:

ID INFORMATION:

SYSTEM: Chesapeake Bay Maryland

BM Gp	Species	Code	Num	*	BM Gp	Species	Code	Num	*
45	Nemertinea	NEMERTIN			24	Pectinaria gouldii	PECTGOUL		
33	Gemma gemma	GEMMGEMM			26	Podarkeopsis levifuscina	PODALEVI		
38	Lyonsia hyalina	LYONHYAL			47	Polychaeta	POLYCHAE		
28	Macoma balthica	MACOBALT			16	Polydora cornuta	POLYCORN		
28	Macoma mitchelli	MACOMITC			26	Pseudeurythoe paucibranchiata	PSEUPAUC		
35	Mulinia lateralis	MULILATE			25	Scolelepis texana	SCOLTEXA		
38	Mya arenaria	MYAAREN			25	Spiophanes bombyx	SPIOBOMB		
38	Parvilucina multilineata	PARVMULT			15	Streblospio benedicti	STREBENE		
34	Rangia cuneata	RANGCUNE			2	Corophium lacustre	COROLACU		
38	Tagelus plebeius	TAGEPLEB			5	Leptocheirus plumulosus	LEPTPLUM		
28	Tellina agilis	TELLAGIL			6	Monoculodes sp. 1 Watling	MONOSPE1		
39	Acteocina canaliculata	ACTECANA			9	Cyathura polita	CYATPOLI		
41	Acteon punctostriatus	ACTEPUNC			10	Edotea triloba	EDOTTRIL		
41	Haminoea solitaria	HAMISOLI			46	Neomysis americana	NEOMAMER		
27	Oligochaeta	OLIGOCHA			44	Phoronis spp.	PHORONIS		
19	Glycera dibranchiata	GLYCDIBR			42	Leptosynapta tenuis	LEPTTENU		
26	Glycinde solitaria	GLYCSOLI							
14	Heteromastus filiformis	HETEFILI							
26	Hypereteone heteropoda	ETEOHETE							
23	Leitoscoloplos robustus	LEITROBU							
23	Leitoscoloplos spp.	LEITOSCO							
18	Marenzelleria viridis	MAREVIRI							
13	Mediomastus ambiseta	MEDIAMBI							
21	Neanthes succinea	NEANSUCC							
17	Paraprionospio pinnata	PARAPINN							

*: Write '1' in this column if taxon is NOT to be considered when counting species in sample

Num=# of Individuals

Number of Biomass Vials

Page ___ of ___

NEAR-COASTAL EMAP 1993
Species Abundance Data Sheet
Southern Polyhaline

EXAMPLE

SAMPLE: EXAMPLE Priority 1 Abundance: Page 1

LAB: Cove Corporation

LAB SAMPLE #:

ID INFORMATION:

SYSTEM: Chesapeake Bay Maryland

BM Gp	Species	Code	Num	*	BM Gp	Species	Code	Num	*
46	Turbellaria	TURBELLA			26	Drilonereis longa	DRILLONG		
45	Nemertinea	NEMERTIN			19	Glycera americana	GLYCAMER		
38	Anadara transversa	ANADTRAN			19	Glycera dibranchiata	GLYCDIBR		
48	Bivalvia	BIVALVIA			19	Glycera spp.	GLYCERA		
36	Ensis directus	ENSIDIRE			26	Glycinde solitaria	GLYCSOLI		
33	Gemma gemma	GEMMGEMM			14	Heteromastus filiformis	HETEFILI		
28	Macoma tenta	MACOTENT			26	Hypereteone heteropoda	ETEOHETE		
35	Mulinia lateralis	MULILATE			23	Leitoscoloplos robustus	LEITROBU		
31	Nucula annulata	NUCUANNU			23	Leitoscoloplos spp.	LEITOSCO		
38	Parvilucina multilineata	PARVMULT			26	Lepidametria commensalis	LEPICOMM		
38	Pitar morrhuanus	PITAMORR			25	Loimia medusa	LOIMMEDU		
28	Tellina agilis	TELLAGIL			25	Magelona spp.	MAGELONA		
28	Tellinidae	TELLINID			20	Maldanidae	MALDANID		
32	Yoldia limatula	YOLDLIMA			13	Mediomastus ambiseta	MEDIAMBI		
39	Acteocina canaliculata	ACTECANA			21	Neanthes succinea	NEANSUCC		
41	Anachis lafresnayi	ANACLAFR			22	Nephtys picta	NEPHPICT		
41	Astyris lunata	ASTYLUNA			17	Paraprionospio pinnata	PARAPINN		
41	Odostomia engonia	ODOSENGO			24	Pectinaria gouldii	PECTGOUL		
41	Turbonilla interrupta	TURBINTE			26	Phyllodoce arenae	PHYLAREN		
27	Oligochaeta	OLIGOCHA			26	Podarkeopsis levifuscina	PODALEVI		
26	Ancistrosyllis hartmanae	ANCIHART			47	Polychaeta	POLYCHAE		
25	Aricidea catherinae	ARICCATH			16	Polydora cornuta	POLYCORN		
25	Asabellides oculata	ASABOCUL			25	Prionospio perkinsi	PRIOPERK		
26	Bhawania heteroseta	BHAWHETE			26	Pseudeurythoe paucibranchiata	PSEUPAUC		
20	Clymenella torquata	CLYMTORQ			26	Sigambra tentaculata	SIGATENT		

*: Write '1' in this column if taxon is NOT to be considered when counting species in sample

Num=# of Individuals

Page ___ of ___

NEAR-COASTAL EMAP 1993
Species Abundance Data Sheet
Southern Polyhaline

EXAMPLE

SAMPLE: EXAMPLE **Priority 1** Abundance: Page 2

SYSTEM: Chesapeake Bay Maryland

BM Gp	Species	Code	Num	*	BM Gp	Species	Code	Num	*
25	Spiochaetopterus costarum	SPIOCOST							
25	Spiophanes bombyx	SPIOBOMB							
15	Streblospio benedicti	STREBENE							
25	Tharyx sp. A Morris	THARSPA							
1	Ampelisca abdita-vadorum cpx	AMPEABVA							
1	Ampelisca verrilli	AMPEVERR							
8	Batea catharinensis	BATECATH							
2	Corophium tuberculatum	COROTUBE							
8	Listriella barnardi	LISTBARN							
8	Listriella clymenellae	LISTCLYM							
7	Unciola serrata	UNCISERR							
46	Balanus improvisus	BALAIMPR							
46	Leucon americanus	LEUCAMER							
46	Oxyurostylis smithi	OXYUSMIT							
11	Ogyrides alphaerostris	OGYRALPH							
11	Pinnixa spp.	PINNIXA							
10	Edotea triloba	EDOTTRIL							
44	Phoronis spp.	PHORONIS							

*: Write '1' in this column if taxon is NOT to be considered when counting species in sample

Num=# of Individuals

Number of Biomass Vials

Page ___ of ___

NEAR-COASTAL EMAP 1993
Species Abundance Data Sheet
Northern Polyhaline

EXAMPLE

SAMPLE: EXAMPLE Priority **1** Abundance: Page 1

LAB: Cove Corporation

LAB SAMPLE #:

ID INFORMATION:

SYSTEM: Chesapeake Bay Maryland

BM Gp	Species	Code	Num	*	BM Gp	Species	Code	Num	*
46	Turbellaria	TURBELLA			19	Glycera dibranchiata	GLYCDIBR		
45	Nemertinea	NEMERTIN			19	Glycera spp.	GLYCERA		
36	Ensis directus	ENSIDIRE			26	Glycinde solitaria	GLYCSOLI		
33	Gemma gemma	GEMMGEMM			26	Goniadidae	GONIADID		
38	Lyonsia hyalina	LYONHYAL			14	Heteromastus filiformis	HETEFILI		
28	Macoma tenta	MACOTENT			23	Leitoscoloplos robustus	LEITROBU		
35	Mulinia lateralis	MULILATE			23	Leitoscoloplos spp.	LEITOSCO		
38	Mytilus edulis	MYTIEDUL			26	Lepidametria commensalis	LEPICOMM		
31	Nucula annulata	NUCUANNU			25	Levinsenia gracilis	LEVIGRAC		
38	Pitar morrhuanus	PITAMORR			25	Loimia medusa	LOIMMEDU		
28	Tellina agilis	TELLAGIL			20	Maldanidae	MALDANID		
32	Yoldia limatula	YOLDLIMA			13	Mediomastus ambiseta	MEDIAMBI		
39	Acteocina canaliculata	ACTECANA			21	Neanthes succinea	NEANSUCC		
41	Anachis lafresnayi	ANACLAFR			26	Nephtyidae	NEPHTYID		
41	Astyris lunata	ASTYLUNA			22	Nephtys incisa	NEPHINCI		
41	Nassarius trivittatus	NASSTRIV			22	Nephtys picta	NEPHPICT		
41	Turbonilla interrupta	TURBINTE			17	Paraprionospio pinnata	PARAPINN		
27	Oligochaeta	OLIGOCHA			24	Pectinaria gouldii	PECTGOUL		
25	Ampharete arctica	AMPHARCT			26	Phyllodoce arenae	PHYLAREN		
25	Ampharetidae	AMPHARTD			26	Podarkeopsis levifuscina	PODALEVI		
25	Aricidea catherinae	ARICCATH			47	Polychaeta	POLYCHAE		
25	Asabellides oculata	ASABOCUL			25	Polycirrus spp.	POLYCIRR		
20	Clymenella torquata	CLYMTORQ			16	Polydora cornuta	POLYCORN		
24	Cossura longocirrata	COSSSOYE			25	Prionospio steenstrupi	PRIOSTEE		
19	Glycera americana	GLYCAMER			26	Sigambra tentaculata	SIGATENT		

*: Write '1' in this column if taxon is NOT to be considered when counting species in sample

Num=# of Individuals

Page ___ of ___

NEAR-COASTAL EMAP 1993
Species Abundance Data Sheet
Northern Polyhaline

EXAMPLE

SAMPLE: EXAMPLE **Priority 1** Abundance: Page 2

SYSTEM: Chesapeake Bay Maryland

BM Gp	Species	Code	Num	*	BM Gp	Species	Code	Num	*
25	Spiochaetopterus costarum	SPIOCOST							
25	Spiophanes bombyx	SPIOBOMB							
15	Streblospio benedicti	STREBENE							
25	Tharyx sp. A Morris	THARSPA							
1	Ampelisca abdita-vadorum cpx	AMPEABVA							
1	Ampelisca verrilli	AMPEVERR							
2	Corophium spp.	COROPHIU							
5	Leptocheirus pinguis	LEPTPING							
7	Unciola irrorata	UNCIIRRO							
7	Unciola spp.	UNCIOLA							
46	Leucon americanus	LEUCAMER							
46	Oxyurostylis smithi	OXYUSMIT							
11	Cancer spp.	CANCER							
11	Crangon septemspinosa	CRANSEPT							
11	Pinnixa spp.	PINNIXA							
10	Edotea triloba	EDOTTRIL							
44	Phoronis spp.	PHORONIS							

*: Write '1' in this column if taxon is NOT to be considered when counting species in sample

Num=# of Individuals

Number of Biomass Vials

Page ___ of ___

NEAR-COASTAL EMAP 1993
Biomass Data Sheet

A EXAMPLE

SAMPLE: EXAMPLE	Priority 1		Biomass: Page 1
LAB: Versar ESM Operations		OLIGO. SPLIT:	CHIRON. SPLIT:
TARE 1 DATE:	BY:	TARE 2 DATE:	BY:
TARE 3 DATE:	BY:	LAB SAMPLE #:	

Vial √	BM Gp	Species	Code	Pan #	Pan Wt (g)	Dry Wt 1 (g)	Dry Wt 2 (g)	Dry Wt U (g)
	1	Ampelisca spp.	AMPELISC					
	2	Corophium spp.	COROPHIU					
	3	Gammaridae	GAMMARID					
	4	Haustoriidae	HAUSTIDA					
	5	Leptocheirus spp.	LEPTOCHE					
	6	Monoculodes spp.	MONOCULO					
	7	Unciola spp.	UNCIOLA					
	8	Amphipoda - Other	AMPHIPOD					
	9	Cyathura spp.	CYATHURA					
	10	Isopoda - Other	ISOPODA					
	11	Decapoda	DECAPODA					
	12	Chironomidae - Fragments	CHRNMDAE					
	49	Chironomidae - Heads						
	13	Mediomastus ambiseta	MEDIAMBI					
	14	Heteromastus filiformis	HETEFILI					
	15	Streblospio benedicti	STREBENE					
	16	Polydora spp.	POLYDORA					
	17	Paraprionospio pinnata	PARAPINN					
	18	Marenzelleria viridis	MAREVIRI					
	19	Glycera spp.	GLYCERA					
	20	Maldanidae	MALDANID					
	21	Neanthes succinea	NEANSUCC					
	22	Nephtys spp.	NEPHTYS					
	23	Leitoscoloplos spp.	LEITOSCO					
	24	Polychaetes - Other: Sub-surface Deposit Feeders	POLYCSUB					
	25	Surf.Depst/Suspensn Feeders	POLYCSUR					
	26	Carnivores/Omnivores	POLYCCAR					

Page ___ of ___

NEAR-COASTAL EMAP 1993
Biomass Data Sheet

B EXAMPLE

SAMPLE: EXAMPLE Priority **1** Biomass: Page 2

LAB: Versar ESM Operations **LAB SAMPLE #:**

Vial #	BM Gp	Species	Code	Pan #	Pan Wt (g)	Dry Wt 1 (g)	Dry Wt 2 (g)	Dry Wt U (g)
	47	Polychates - Unident + frags.	POLYCHAE					
	27	Oligochaeta - Fragments	OLIGOCHA					
	50	Oligochaeta - Heads						
	28	Tellinidae	TELLINID					
	29	Corbicula fluminea	CORBFLUM					
	30	Mercenaria mercenaria	MERCMERC					
	31	Nucula spp.	NUCULA					
	32	Yoldia limatula	YOLDLIMA					
	33	Gemma gemma	GEMMGEMM					
	34	Rangia cuneata	RANGCUNE					
	35	Mulinia lateralis	MULILATE					
	36	Ensis directus	ENSIDIRE					
	37	Bivalvia: Other - Deposit Feeders	BIVALDEP					
	38	- Suspension Feeders	BIVALSUS					
	48	- Unidentified	BIVALVIA					
	39	Acteocina canaliculata	ACTECANA					
	40	Hydrobia spp.	HYDROBIA					
	41	Gastropoda - Other	GASTROPO					
	42	Echinodermata	ECHINODE					
	43	Hemichordata	HEMICHOR					
	44	Phoronis spp.	PHORONIS					
	45	Nemertinea	NEMERTIN					
	46	Miscellanea	MISCELLA					

Number of Biomass Vials ____

Page ___ of ___

NEAR-COASTAL EMAP 1993
Biomass Data Sheet

A EXAMPLE

SAMPLE: EXAMPLE Priority 1	Biomass: Page 1
LAB: Versar ESM Operations	**LAB SAMPLE #:**
TARE 1 DATE: **BY:**	**TARE 2 DATE:** **BY:**
TARE 3 DATE: **BY:**	

Vial /	BM Gp	Species	Code	Pan #	Pan Wt (g)	Dry Wt 1 (g)	Dry Wt 2 (g)	Dry Wt U (g)
	1	Ampelisca spp.	AMPELISC					
	2	Corophium spp.	COROPHIU					
	3	Gammaridae	GAMMARID					
	4	Haustoriidae	HAUSTIDA					
	5	Leptocheirus spp.	LEPTOCHE					
	6	Monoculodes spp.	MONOCULO					
	7	Unciola spp.	UNCIOLA					
	8	Amphipoda - Other	AMPHIPOD					
	9	Cyathura spp.	CYATHURA					
	10	Isopoda - Other	ISOPODA					
	11	Decapoda	DECAPODA					
	12	Chironomidae	CHRNMDAE					
	13	Mediomastus ambiseta	MEDIAMBI					
	14	Heteromastus filiformis	HETEFILI					
	15	Streblospio benedicti	STREBENE					
	16	Polydora spp.	POLYDORA					
	17	Paraprionospio pinnata	PARAPINN					
	18	Marenzelleria viridis	MAREVIRI					
	19	Glycera spp.	GLYCERA					
	20	Maldanidae	MALDANID					
	21	Neanthes succinea	NEANSUCC					
	22	Nephtys spp.	NEPHTYS					
	23	Leitoscoloplos spp.	LEITOSCO					
	24	Polychaetes - Other: Sub-surface Deposit Feeders	POLYCSUB					
	25	Surf.Depst/Suspensn Feeders	POLYCSUR					
	26	Carnivores/Omnivores	POLYCCAR					
	47	Unidentified (inc fragmnts)	POLYCHAE					

Page ___ of ___

NEAR-COASTAL EMAP 1993
Biomass Data Sheet

B EXAMPLE

SAMPLE: EXAMPLE	Priority 1	Biomass: Page 2
LAB: Versar ESM Operations	**LAB SAMPLE #:**	

Vial √	BM Gp	Species	Code	Pan #	Pan Wt (g)	Dry Wt 1 (g)	Dry Wt 2 (g)	Dry Wt U (g)
	27	Oligochaeta	OLIGOCHA					
	28	Tellinidae	TELLINID					
	29	Corbicula fluminea	CORBFLUM					
	30	Mercenaria mercenaria	MERCMERC					
	31	Nucula spp.	NUCULA					
	32	Yoldia limatula	YOLDLIMA					
	33	Gemma gemma	GEMMGEMM					
	34	Rangia cuneata	RANGCUNE					
	35	Mulinia lateralis	MULILATE					
	36	Ensis directus	ENSIDIRE					
	37	Bivalvia: Other - Deposit Feeders	BIVALDEP					
	38	- Suspension Feeders	BIVALSUS					
	48	- Unidentified	BIVALVIA					
	39	Acteocina canaliculata	ACTECANA					
	40	Hydrobia spp.	HYDROBIA					
	41	Gastropoda - Other	GASTROPO					
	42	Echinodermata	ECHINODE					
	43	Hemichordata	HEMICHOR					
	44	Phoronis spp.	PHORONIS					
	45	Nemertinea	NEMERTIN					
	46	Miscellanea	MISCELLA					

Number of Biomass Vials ____

Data Form - Laboratory Sample Tracking Form

Laboratory Sample Tracking Form							
Project_____				Collection Date_____			
Station Replicate Serial No.	Sorting*		General ID **	Oligochaete Preparation **	Chironomid Preparation**	Chironomid ID **	QC**
	Personnel	Vials Generated					

* Initials, date, time estimate, abbreviated taxa name of vial constituents
** Initials, date, time estimate

Data Form - QC SAMPLE BATCH LISTING FORM

Sorter/Taxonomist _____

Batch For _____
Sample Month

1	11	21	31
2	12	22	32
3	13	23	33
4	14	24	34
5	15	25	35
6	16	26	36
7	17	27	37
8	18	28	38
9	19	29	39
10	20	30	40

Necessary Remedial Action:

_____

_____

_____

Comments:

_____

_____

_____

Data Form - **QC SAMPLE RESORT SHEET**

Project _____ Station/Rep _____

Collection Date_____ Original Sorter_____

Additional Organisms Found			
Taxa	**Number**	**Taxa**	**Number**

Total organisms originally sorted _____

Total additional organisms found _____

% error _____

Resorted by _____

QC OK'd by _____

Any remedial action necessary:

_____

_____

_____

Comments:

_____

_____

_____

Data Form - **QC SAMPLE REIDENTIFICATION SHEET**

Project _____ Collection Date _____

Station _____ Original Processor _____

Taxon	QC Recount	Original count	Taxon	QC Recount	Original Recount
			Total Counts		

No. of Miscounts (- I Recount - original count) _____

No. of Misidentifications _____

Total No. of Errors _____

$$\% \text{ Error} = \frac{\text{Total No. Original Inds.} - \text{Total No. Errors}}{\text{Total No. Inds.}}$$

QC'd by _____ Date _____

Other problems with Sample:

Individual placed in wrong vial _____

Counts records on wrong line _____

Inadequate labels _____

Other _____

Data Form - BIOMASS LOG SHEET

BIOMASS LOG

Batch Number	Sample ID	Date Pan Weight	Date First Biomass Weight	Date Second Biomass Weight	Initials Complete

QC Biomass Reweighing and Blanks

	Batch Number

Blanks

	Crucible Number	Crucible weight	Dr_2 Weight #1	D_2 Weight #2

Quality Control

	Crucible Number	Original Weight	Reweigh Weight	Initials

SECTION 4

HISTOPATHOLOGY

1. INTRODUCTION

1.1 Fish quality in EMAP-Estuaries will be measured as a composite index of the incidence of diseases and contaminant body burdens in selected resident species. Both of these factors are key elements of the public perception of the environmental quality of estuarine habitats. The assessment and improvement of fish quality relates directly to the Clean Water Act goal of protection and maintenance of fishable waters. While gross fish pathology is a potential response indicator of environmental status that is easy and economical to measure, it may not provide insight into the potential cause of the pathological abnormalities or may not be related to environmental quality. To address this concern, EMAP-Estuaries will perform detailed histopathological examinations of randomly selected individuals of target and non-target fish species at the indicator testing and evaluation sites. All individuals of each target species that "fail" the field gross pathology examination and up to 25 randomly selected individuals of each target species that "pass" the field examination at the indicator testing and evaluation sites will undergo a detailed gross and histopathological examination. In addition up to ten randomly selected individuals from non-target species collected at these sites will be examined similarly. Detailed histopathology exams will also be conducted on collected fish that have gross pathological disorders. The results of this microscopic examination will be used to assess the relationship between the incidence of external abnormalities and internal histopathological abnormalities, to characterize the types of external/internal pathologies and to create a baseline of histopathological information for the Virginian and Louisianian Provinces.

2. LABORATORY EXAMINATION OF FISH

2.1 The laboratory examination of finfish for pathological abnormalities will be conducted using two somewhat different procedures corresponding to the two types of field sampling (*i.e*., base sampling and indicator validation sampling) conducted during the Near Coastal EMAP Virginian Pilot Demonstration. The corresponding pathology methods are described below.

2.2 Base Samples

2.2.1 Fixed specimens will be unpacked, logged in, and placed in 70% ethyl alcohol (EtOH) for at least 48 hours prior to examination. The specimens from the Base Sampling sites will be subjected to a critical gross examination as described below and findings will be compared to the findings from the field examination.

2.2.1.1 A careful visual inspection will be made of the fins and body surfaces. Any discolorations of body surfaces, hemorrhaging, raised scales, white spots, parasites visible to the naked eye, lumps, bumps, or

other growths, ulcerations, fin erosion, deformities of the vertebral column and/or mandibles, swelling of the anus, or any other abnormal conditions will be noted.

2.2.1.2 Eyes will be examined noting any hemorrhage, exophthalmia (*i.e.*, pop eye), microphthalmia (*i.e.*, depression into the orbits), or cataracts.

2.2.1.3 A thorough examination of the branchial chamber and buccal cavity will be done identifying any pathological abnormalities such as opercular perforations or deformities, lumps, bumps, ulcerations, gill erosion, clubbing or other deformities, and/or parasitic infestations.

2.2.1.5 A thorough gross examination of the visceral organs will be performed and any abnormalities will be noted.

2.2.2 Representative tissue samples from any of the gross pathologies will be taken and placed in properly labeled tissue-processing cassettes for possible future evaluation.

2.3 Indicator Validation Samples

2.3.1 Specimens from the Indicator Validation sites will be examined grossly as described for the Base Samples.

2.3.2 Representative tissue samples from any noted gross pathologies will be taken and placed in properly labeled tissue-processing cassettes. All tissue specimens of gross pathologies will be processed for routine paraffin histological evaluation.

2.3.3 Tissue samples of liver and spleen will be taken from each specimen from Indicator Validation sites and placed in properly labeled tissue-processing cassettes. All tissue specimens will be processed for routine paraffin histological evaluation.

3. HISTOPATHOLOGICAL PROCESSING

3.1 All tissue specimens selected for histological evaluation will be placed in properly labeled tissue-processing cassettes.

3.2 Tissue specimens in labeled cassettes will be dehydrated, cleared, and infiltrated with Paraplast® X-TRA using an automated tissue processor (Shandon Hypercenter® 2 Tissue Processing System). The processing schedule is as follows:

Step	Reagent	Temp	Vacuum	Immersion Time	Drain Time*
1	70% EtOH	A	N	01:00:00	120
2	80% EtOH	A	N	01:00:00	120
3	95% EtOH	A	N	01:00:00	120
4	95% EtOH	A	N	01:00:00	120
5	100% EtOH	A	N	01:00:00	120
6	100% EtOH	A	N	00:50:00	120
7	100% EtOH	A	N	00:50:00	120
8	xylenes	A	N	01:00:00	120
9	xylenes	A	N	01:00:00	120
10	xylenes	A	Y	01:00:00	120
11	Paraplast	60	Y	01:00:00	120
12	Paraplast	60	Y	01:00:00	120

* Seconds
A Ambient
Y Yes
N No

3.3 Tissue specimens will be embedded in Paraplast® X-TRA. Labeled tissue-processing cassettes are used in the embedding process so that the tissue sample and cassette are molded together with the hardened Paraplast® X-TRA thus retaining the processing code with the tissue sample.

3.4 Representative sections will be cut from each tissue specimen using a rotary microtome. Sections will be cut at 6 μm and then floated on a waterbath containing 6 ml of Surgipath® Stayon. Sections will be collected on slides labeled with the appropriate processing code and then placed in a drying oven (48°C) overnight. Two slides containing two to five sections each will be prepared from each specimen.

3.5 Slides will be stained with Harris' hematoxylin and eosin for routine histological examination. The staining schedule utilized is as follows:

Station	Reagent	Time
1	Xylenes	3 min.
2	Xylenes	3 min.
3	Xylenes	3 min.
4	100% EtOH	1 min.
5	100% EtOH	1 min.
6	95% EtOH	1 min.
7	70% EtOH	1 min.
8	Running Water	1 min.
9	Hematoxylin	9 min.
10	Running Water	1 min.
11	Acid Alcohol	1 sec.
12	Running Water	1 min.
13	Ammonia Water	1 min.
14	Running Water	1 min.
15	70% EtOH	1 min.
16	Eosin Y	5 sec.
17	95% EtOH	5 sec.
18	95% EtOH	1 min.
19	100% EtOH	1 min.
20	100% EtOH	1 min.
21	100% EtOH	1 min.
22	Xylenes	1 min.
23	Xylenes	1 min.
24	Xylenes	

Following staining, slides will be cover-slipped using 24 x 50 mm or 24 x 55 mm, No. 1 ½ coverslips and Permount®, and dried overnight in a drying oven.

4. HISTOLOGICAL EXAMINATION

4.1 Slides will be examined using a compound research microscope. Notes on the condition of the tissue specimens will be recorded on data sheets. Diagnoses of pathologic conditions will be made and the coded data will be entered into a database management program for subsequent transfer to the EMAP Near Coastal database. Diagnostic codes will consist of three parts, a condition identification (Table 1), a tissue site identification (Table 2), and , where appropriate, an intensity identifier (i.e., 1 - 4 with 4 being the most severe condition).

Table 1. Diagnostic Codes Used for Histopathologic Examination of EMAP Specimens*

DIAGNOSIS	CODE
No pathologic changes	NPC
GENERAL PATHOLOGIC ABNORMALITIES	
Traumatic injury	TI
Granulomatous inflammation	GI
Inflammatory focus (foci)	IF
Fatty degeneration	FD
Autolysis	AL
Congestion	C
Dysplasia	DY
Enteritis	E
Necrosis	N
Hyperplasia	H
Megalocytosis	M
Fibrosis	FB
INFECTIOUS DISEASES	
Lymphocystis	L
Bacterial infections	BI
Fungal infections	FU

(continued)

* This list of coded diagnoses is by no means a complete listing of possible conditions and may be amended as necessary.

Table 1. continued.

DIAGNOSIS	CODE
PARASITIC DISEASES	
Protozoans	PZ
Monogeneans	MG
Digeneans	DG
Cestodes	CT
Nematodes	NT
Aeanthocephalans	AT
Crustaceans	CR
MISCELLANEOUS RESPONSES	
Ductal proliferation	DP
Rodlet cell response	RCR
Spongiosis hepatis	SH
Spongiosis, kidney	KS
PRENEOPLASTIC LIVER LESIONS	
Vacuolated cell focus (foci)	VCF
Clear cell focus (foci)	CCF
Eosinophilic focus (foci)	EF
Basophilic focus (foci)	BF
NEOPLASTIC LESIONS	
LIVER	
Adenoma	A
Hepatocellular carcinoma	HC
Cholangioma	CL
Cholangiocellular carcinoma	CC
Pericytoma	PC

(continued)

Table 1. continued.

DIAGNOSIS	CODE
PANCREAS	
Atypical acinar cell focus (foci)	AAFC
Acinar cell adenoma	ACA
Acinar cell carcinoma	ACC
Adenocarcinoma	ADC
CARDIOVASCULAR	
Hemangioma	HM
Hemangioendothelioma	HE
Hemangioendotheliosarcoma	HES
Hemangiopericytoma	HP
BONE AND CARTILAGE	
Hyperostosis	HO
Osteoma	O
Osteochondroma	OC
Osteosarcoma	OS
Chondroma	CD
Chondrosarcoma	CS
SOFT TISSUES	
Lipoma	LP
Liposarcoma	LPS
Fibroma	F
Fibrosarcoma	FS
Leiomyoma	LM
Leiomyosarcoma	LMS
Rhabdomyoma	RM
Rhabdomyosarcoma	RMS
Mesothelioma	MT

(continued)

Table 1. continued.

DIAGNOSIS	CODE
KIDNEY AND URINARY TRACT	
Polycystic kidney	PK
Nephroblastoma	NB
Renal adenocarcinoma	RA
HEMATOPOIETIC TISSUES	
Lymphoma	LY
Malignant fibrous histiocytoma	MFH
GASTROINTESTINAL TRACT	
Adenoma	AD
Adenocarcinoma	IA
NEURAL	
Neurofibroma	NF
Schwannoma	SW
INTEGUMENT	
Papilloma	PL
Melanoma	ME
Squamous cell carcinoma	SCC

Table 2. Tissue Codes Used for Histopathologic Examination of EMAP Specimens.

TISSUE	CODE	TISSUE	CODE
Barbel, mandibulary	BD	Gonad	GO
Barbel, maxillary	BM	Gut	GT
Barbel, mental	BB	Heart	HT
Barbel, nasal	BN	Kidney	KD
Bile duct	BI	Kidney, head	KH
Blood	BL	Kidney, trunk	KT
Blood vessel	BV	Kidney interstitium	KI
Body (trunk), xsec	BX	Liver	LV
Bone	BO	Liver surface	LS
Brain	BR	Meninges	MN
Buccal cavity	BU	Mesentery	ME
Cartilage	CA	Mouth	MO
Caudal peduncle	CP	Muscle	MS
Dermis (epidermis)	DM	Nares	NA
Dermis (site 1)	D1	Ovary	OV
Dermis (site 2)	D2	Pancreas	PA
Dermis (site 3)	D3	Pseudobranch	PS
Eye	EY	Pyloric caeca	PC
Fin, anal	AF	Renal tubules	RT
Fin, caudal	CF	Skin	SK
Fin, dorsal	DF	Spleen	SP
Fin, pectoral	PF	Swimbladder	SW
Gall bladder	GB	Testis	TE
Gills	GI	Thymus	TH
Gill arch	GL	Thyroid	TY
Gill arch, right	G1	Visceral mass	VM
Gill arch, left	G2		

SECTION 5

SEDIMENT SILT-CLAY CONTENT
SEDIMENT GRAIN SIZE DISTRIBUTION
TOTAL ORGANIC CARBON CONCENTRATION
LABORATORY PROCEDURES

TABLE OF CONTENTS

Subsections

1. INTRODUCTION

1.1 Habitat indicators provide important information about the environmental setting of a sample site. Salinity and temperature are among the most important factors controlling the distribution of biota and ecological processes in estuaries. Organic content and grain size distribution are major sediment characteristics that influence sediment quality and processes, as well as benthic invertebrate distributions. Cumulatively, these parameters define the major habitats sampled by EMAP-Estuaries and information on these habitat indicators will be essential for normalizing responses of exposure and response indicators to natural environmental gradients. They will also be used to define subpopulations for analysis and integration activities.

1.2 This chapter describes the laboratory procedures used to determine the silt-clay content, water content, grain size distribution, and total organic carbon concentration of sediment collected for EMAP-Estuaries. The procedures are designed to yield reliable, reproducible results and incorporate specific quality assurance/quality control procedures.

1.3 The laboratory procedures are based upon currently accepted practices in benthic ecology and sedimentology (Buchanan 1984; Plumb 1981). Although these practices are fairly standard throughout the research community, certain procedures may require modification during a long-term (decades) program such as EMAP. Such modifications will be kept to a minimum to ensure the long-term comparability of data and will not occur without prior consultation with the Near Coastal Technical Director. Modifications will be incorporated into this manual in a timely manner.

2. LABORATORY SAFETY

2.1 Safe laboratory procedures must be followed at all times and are outlined in a laboratory safety manual which is posted in the laboratory.

2.2 Eye protection (*i.e.*, laboratory safety glasses or approved prescription glasses) must be worn when handling chemicals or when performing procedures that require use of a fume hood. Safety glasses are available in the laboratory.

2.3 Respirators capable of filtering small particles must be used when grinding sediments that may contain high concentrations of chemical contaminants.

2.4 No chemicals are to be poured down laboratory drains connecting to public or private sewer systems. Exceptions to this rule depend upon local and state regulations concerning the disposal of hazardous wastes.

2.5 All work areas are to be kept clean and neat at all times.

2.6 Any conditions hindering accomplishment of work or presenting a safety hazard should be brought to the attention of the laboratory supervisor immediately.

3. TRAINING

3.1 A program the size of EMAP involves many field and laboratory technicians. To ensure that data produced by all workers are comparable, standard procedures must be followed.

3.2 All personnel will have a minimum level of training. Instruction will include evaluation and proficiency testing to insure the mastery of basic skills.

3.3 Training will be provided by experienced personnel in established laboratories. New employees will learn laboratory techniques using practice samples and, will not handle real EMAP samples without supervision and demonstration of acceptable proficiency.

3.4 The overall proficiency of laboratory personnel will be evaluated using the various QA/QC procedures outlined in the methods described below. The status of personnel demonstrating substandard performance will be reevaluated, and personnel will be retrained or removed from the laboratory, if necessary to maintain consistent, high quality production of data.

4. SAMPLE STORAGE AND PROCESSING

4.1 Sediment samples may be chilled at 4 to 5 °C prior to processing, but samples should not be allowed to dry before grain size analyses are conducted (Plumb, 1981).

4.2 Sieves used in the determination of sediment grain size will not be used for other purposes (*e.g.*, benthic sorting). All wet sieving procedures are to be carried out using stainless steel screens. Fine screens (63 μm mesh) will be cleaned with copious amounts of water to prevent clogging of mesh openings. Screens will not be cleaned with brushes, which may distort openings. Sediments will not be forced through screens.

4.3 An analytical balance accurate to 0.1 mg will be used for all weighing. Prior to each use, the balance will be zeroed, and its calibration will be checked using a standard weight. The same standard weight (each standard is numbered) will be used for all weight measurements for a particular batch of samples.

5. PROCEDURES FOR SILT-CLAY CONTENT DETERMINATION

5.1 The following procedures from Folk, 1968; Lewis, 1984; and Lewis and McConchie, 1994 are used to determine the percent by weight of silts and clays in sediment samples. Silts and clays are those particles that pass through a 63 μm mesh sieve. Materials retained on the sieve used in this procedure are generally sands (>63 μm but < 2 mm) but may include gravel sized particles (> 2mm but < 64mm -- size classification according to the Wentworth-Lane scale, Pettijohn 1975).

5.2 Sediment samples will be retrieved from cold storage and brought to room temperature. Sample numbers will be recorded on a silt-clay analysis data sheet upon retrieval from storage.

5.3 Sediments will be removed from storage bags, placed in a clean 250 ml glass beaker and homogenized. Homogenization will be accomplished by stirring the sediment with a small spatula with a small amount of deionized water added for lubrication (if necessary) for at least three minutes. After stirring, rinse sediment from the spatula back into beaker using deionized water.

5.4 How Much Sediment to Use For Analysis?

5.4.1 The amount of sediment to be processed depends upon sediment type. The technician will visually classify samples as primarily sand or primarily mud based upon the texture of sediments.

5.4.2 The best amount of sample for processing is approximately 15-20 grams of mud (*i.e.*, sample in the < 63 μm fraction). With more sample, the grains interfere with each other too much during settling and may flocculate; with too little sample, the experimental error in weighing becomes large with respect to the sample size.

5.4.3 For sandy sediments, approximately 45-50 g wet weight will be removed from the 250 ml glass beaker and placed into a clean 100 ml glass beaker for wet sieving. Note the importance of Section 5.5 because of the coarseness of the sample.

5.4.4 For muddy sediments, approximately 20-25 g wet weight will be removed from the 250 ml glass beaker to a 100 ml glass beaker for wet sieving.

5.4.5 The remaining sediment will be returned to the original storage bag and held in cold storage until all QA/QC checks for this sample have been passed.

5.5 Dispersion of Clay Fraction of Sediments:

5.5.1 Make-up a 5g/L stock solution of dispersant. Add 5 grams of sodium hexametaphosphate "Calgon" to 1 liter of deionized water.

5.5.2 Add 20 ml of the dispersant solution (100 mg of hexametaphosphate) and 30 ml of distilled water to the sample. Stir, using a magnetic stirrer for one to five minutes to break-up sediment aggregrates.

5.6 Wet Sieving the Sample:

5.6.1 After stirring, the sample will be wet sieved through a 63 μm mesh sieve into a large evaporation dish using as little distilled water as possible.

5.6.2 Place the sieve over the large evaporation dish and wash all fines into the sieve using as little distilled water as possible.

5.6.3 The volume of sediment + water in the evaporation dish must be < 900 ml to allow for rinsing the sample into a 1000 ml graduated cylinder.

5.7 Analysis of the Silt and Clay Fraction (Particles < 63 μm)

5.7.1 Carefully transfer the mud in the evaporation dish to a 1000 ml graduated cylinder. Carefully rinse the mud (generally medium-coarse silt-size (16-63 µm particles) found at the bottom of the dish into the graduated cylinder using deionized water, being careful not to exceed the 1000 ml mark. See Section 5.8 for handling the >63 µm fraction that remains on the sieve.

5.7.2 Fill, with deionized water, up to the 1000 ml mark. Using a metal stirring rod, vigorously stir the water column from bottom to top, using short strokes, starting at the base of the column and working upwards. Keep stirring until the material is distributed uniformly throughout the column. End up stirring with long,smooth strokes the full length of the column. Be careful not to break the water surface as material could be lost. Place a beaker with tap water next to the cylinder and insert a thermometer to record water temperature.

5.7.3 Immediately (20 sec) after stirring, withdraw 40 ml of sample using a 40 ml volumetric pipette. Expel sample into a tared 50 ml glass beaker. Rinse pipette with a small volume of deionized water, and add the rinse to the 50 ml beaker. If the sample is taken in two parts (*i.e*., two 20-ml samples), the cylinder will be stirred between extractions and samples withdrawn after each stirring and added to the beaker.

5.7.4 The 50 ml glass beaker will be placed in an oven at 100°C until dry. Typically, 24 hours is sufficient for sediment samples to come to a stable dry weight. A randomly selected subsample of each batch will be reweighed after an additional 24 hour drying period, as a check for the stability of the dry weight measurement.

5.8 Treatment of >63 µm Fraction Retained on Sieve:

5.8.1 If necessary, remove shell and shells fragments, pieces of wood and algae. Place in plastic weigh pans to air dry. Record weights. Note that these weights are not part of the sand or silt/clay fraction but are part of the sediment record.

5.8.2 The fraction retained on the sieve (>63 μm) will be transferred to a tared 50 ml glass beaker and placed in an oven at 100 °C until dry. Typically, 24 hours is sufficient for the dry weight of sediment samples to stabilize. All samples will be weighed after 24 hours; the weight will be recorded on the biomass data sheets, and the samples will be returned to the drying oven. A randomly selected subsample of each batch will be reweighed after an additional 24-hour drying period, as a check for the stability of the dry weight measurement.

5.9 Storage of Unused Samples:

5.9.1 Unused sediment from each sample will be stored at 4 to 5 °C for QA/QC analyses and other sediment analyses.

5.10 Weighing Samples:

5.10.1 After drying, remove beaker from the oven and let equilibrate with the atmosphere for at least 1.5 hr before weighing. Weigh to nearest 0.001 grams and record weight.

5.11 Calculations for Sand and Silt-Clay (Mud) Determinations:

5.11.1 Sand weight calculation:

$$Sand\ wt. = Gross\ wt.\ (sample + beaker) - tare\ wt.\ (beaker)$$

5.11.2 Silt-clay (mud) weight calculation:

$$Silt\text{-}clay\ wt. = \left[(Gross\ wt. - beaker\ wt.)\ x\ \frac{(total\ volume\ in\ cylinder)}{(sample\ volume\ from\ cylinder)}\right] - \begin{matrix} dispersant \\ weight \end{matrix}$$

Note: The total volume in cylinder is 1000 ml. The sample volume from cylinder is 40 ml. Using the prescribed methods, this results in a dispersant weight of 4 mg.

5.11.3 Percent silt-clay (mud) calculation:

$$\%\ silt\text{-}clay = \left(\frac{silt\text{-}clay\ wt.}{sand\ wt. + silt\text{-}clay\ wt.}\right)\ x\ 100$$

5.11.4 Percent sand calculation:

% sand = 100 - %mud

Note: (100 - % mud) is not, in all cases, equal to the percent sand, since gravel sized particles (> 2mm but < 64 mm) may be present in some samples.

6. PROCEDURES FOR PERCENT WATER CONTENT

6.1 The following procedures are used to determine the percent by weight of water in sediment samples. The percent water content of sediment samples is needed to correct sediment dry weights for salt content, since salts are left behind in the drying process.

6.2 Sample Retrieval:

6.2.1 Sediment samples will be retrieved from cold storage and brought to room temperature. Sample numbers will be recorded on a data sheet upon retrieval from storage. Samples that have dried cannot be processed, since dried sediments yield erroneous results.

6.3 Sample Preparation:

6.3.1 Sediments will be removed from storage bags, placed in a clean 250 ml glass beaker and homogenized. Homogenization will be accomplished by stirring sediment with a small spatula for at least three minutes. **Do not rinse sediment from the spatula into the beaker and do not add water to the beaker during the homogenization process!**

6.3.2 Approximately 5-10 grams wet weight of sediment will be placed in a clean, tared 50 ml glass beaker (or 3.3 ml assuming a wet weight density of 1.5).

6.4 Recording Sediment Weight:

6.4.1 Weigh sample immediately. The sample must not be allowed to stand for more than a few minutes, since evaporation at room temperature will affect the percent water content measurement.

6.5 Drying the Sample:

6.5.1 The sample will be placed in a drying oven at 100°C until dry. Typically, 24 hours is sufficient for the dry weight of sediment samples to stabilize. Dry samples will be stored in a desiccator containing a hydrous silica gel until cooled to touch (approximately 1 hr). (**Note**: Dry sediment samples may absorb moisture from wet sediment samples, thus, dry samples should be removed before placing moist samples in the oven). All weights will be recorded on data sheets to the nearest 0.001 grams. Ten percent (10%) of randomly selected subsamples of each batch will be reweighed after an additional 24-hour drying period, as a check for stability for the dry weight measurement (a change of less than 0.1% is expected).

6.6 Disposal of Unused Sediment

6.6.1 Unused sediment from each sample will be stored at 4 to 5°C for QA/QC analyses and other sediment analyses.

6.7 Calculation of Sediment Water Content:

6.7.1 The following procedures will be used to calculate the water content of sediment. Because dry salts are included in the dry sediment weight, a correction must be applied to account for this (section 6.8.3).

6.7.2 Sediment water content calculation:

$$\%\ water = \left(\frac{(Gross\ wet\ wt. - tare) - (Gross\ dry\ wt. - tare)}{Gross\ wet\ wt. - tare} \right) x\ 100$$

6.7.3 Correction of dry weight for salt content.

6.7.3.1 Equations:

$$Corrected\ dry\ weight = (Gross\ Dry\ wt. - tare) - (Salt\ weight)$$

$$Salt\ weight\ (g) = Water\ loss\ (ml)\ x\ Salinity\ (mg/ml\ [= ‰ = ppt\ ])$$

$$Water\ loss\ (ml) = Gross\ wet\ weight\ (wet\ sample + pan) - Gross\ dry\ weight\ (dry\ sample + pan)$$

assuming a water density of 1 g/ml for the water that evaporates (fresh water)

6.7.3.2 Example calculation of corrected dry weight:

Sample salinity = 12.4 ‰ (as a first approximation, the salinity of interstitial water is assumed to be equal to the salinity of bottom water as measured by EMAP CTD casts).

Beaker Weight = 31.144 g

Wet Sediment + Beaker Weight (Gross Wet Wt) = 39.219 g

Dry Sediment + Beaker Weight = 37.135 g

Known: Water Density = 1g/ml

Calculations:

Water Loss (ml) = *(Wet Sdmt Wt + Bkr Wt) - (Dry Sdmt Wt + Bkr Wt)*
= *39.219 g - 37.135 g*
= *2.084 g*
= *2.084 ml*

Salt Weight (g) = *Water Loss (ml) x Salinity (mg/ml)*
= *2.084 ml x 12.4 mg/ml*
= *25.84 mg*
= *.0258 g*

Corrected Dry Weight (g) $= ((\text{Dry Sdmt} + \text{Bkr Wt}) - \text{Bkr Wt}) - \text{Salt Weight}$
$= (37.135\ g - 31.144\ g) - 0.0258\ g$
$= 5.991\ g - 0.0258\ g$
$= 5.965\ g$

Corrected Percent Water

$$= \frac{((\text{Wet Sdmt} + \text{Bkr Wt}) - \text{Bkr Wt}) - \text{Corrected Dry Wt.}}{((\text{Wet Sdmt} + \text{Bkr Wt}) - \text{Bkr Wt})} \times 100$$

$$= \frac{(39.219 - 31.144) - 5.965}{39.219 - 31.144} \times 100 = 26$$

7. PROCEDURES FOR SEDIMENT GRAIN SIZE DISTRIBUTION DETERMINATION

7.1 The following procedures from Folk, 1968; Lewis, 1984; and Lewis and McConchie, 1994 are used to determine the percent by weight of silts and clays in sediment samples. Silts and clays are those particles that pass through a 63 μm mesh sieve. Materials retained on the sieve used in this procedure are generally sands (>63 μm but < 2 mm) but may include gravel sized particles (> 2mm but < 64mm -- size classification according to the Wentworth-Lane scale, Pettijohn 1975). The procedures allow determination of weight percent quantiles for sediments, as well as the quantile deviation of skewness.

7.2. Sediment samples will be retrieved from cold storage and brought to room temperature. Sample numbers will be recorded on a sediment grain size data sheet upon retrieval from storage.

7.3 How Much Sediment to Use For Analysis?

7.3.1 The best amount of sample for processing is approximately 15-20 grams of mud (*i.e.*, sample in the < 63 μm fraction). With more sample, the grains interfere with each other too much during settling and may flocculate; with too little sample, the experimental error in weighing becomes large with respect to the sample size.

7.3.2 For sandy sediments, approximately 45-50 g wet weight will be removed from the 250 ml glass beaker and placed into a clean 100 ml glass beaker for wet sieving. Note the importance of Section 5.5 because of the coarseness of the sample.

7.3.3 For muddy sediments, approximately 20-25 g wet weight will be removed from the 250 ml glass beaker to a 100 ml glass beaker for wet sieving.

7.3.4 The remaining sediment will be returned to the original storage bag and held in cold storage until all QA/QC checks for this sample have been passed.

7.4 Sample Handling:

7.4.1 Sediments will be removed from storage bags, placed in a clean 250 ml glass beaker, and homogenized. Homogenization will be accomplished by stirring sediment with a spatula and a small amount of deionized water for at least three minutes.

7.5 Removal of Organic Matter for the Determination of "True" Particle Size Distribution (Lewis, 1986):

7.5.1 If the sample is equal to or less than 20% mud (silt-clay), proceed to Section 7.6

7.5.2 If the sample is greater than 20% mud, the organics in the sample must be removed.

7.5.3 Initially, add enough deionized water to cover the sample. Add small quantities of 30% H_2O_2 to the sample, stirring until any effervescence ceases. Cover beaker with large watch glass cover if frothing is excessive. If the solution heats excessively, cool the beaker in a water bath. Continue adding H_2O_2 until frothing ceases, then slowly heat to 60-70°C (H_2O_2 decomposes above 70°C). Observe for 10 mins. to ensure that the possibility of a strong reaction has passed. Add H_2O_2 until no further reaction occurs.

7.6 Dispersion of the Clays

7.6.1 Make-up a stock solution of dispersant. Add 5 grams of sodium hexametaphosphate "Calgon" to 1 liter of deionized water.

7.6.2 Add 20 ml of the dispersant solution (100 mg of hexametaphosphate) and 30 ml of distilled water to the sample. Stir, using a magnetic stirrer for one to five minutes to break-up sediment aggregrates.

7.7 Wet Sieving the Sample:

7.7.1 After stirring, the sample will be wet sieved through a 63 μm mesh sieve into a large evaporation dish using as little distilled water as possible.

7.7.2 Place the sieve over the large evaporation dish and wash all fines into the sieve using as little distilled water as possible.

7.7.3 The volume of sediment + water in the evaporation dish must be < 900 ml to allow for rinsing the sample into a 1000 ml graduated cylinder.

7.7.4 Carefully transfer the mud in the evaporation dish to a 1000 ml graduated cylinder. Carefully rinse the mud (generally medium-coarse silt-size (16-63) µm particles) found at the bottom of the dish into the graduated cylinder using deionized water, being careful not to exceed the 1000 ml mark. See Section 7.9 for handling the sand-size and greater fraction that remains on the sieve (>63 µm fraction).

7.7.5 Fill, with deionized water, up to the 1000 ml mark. Using a metal stirring rod, vigorously stir the water column from bottom to top, using short strokes, starting at the base of the column and working upwards. Keep stirring until the material is distributed uniformly throughout the column. End up stirring with long,smooth strokes the full length of the column. Be careful not to break the water surface as material could be lost. Place a beaker with tap water next to the cylinder and insert a thermometer to record water temperature.

7.8 Analysis of the Silt and Clay (< 63 µm) Fraction :

7.8.1 Stir the cylinder to suspend the sample in accordance with procedures in Section 7.7.5. As soon as the stir rod emerges for the last time, start the timer. At the end of 20 seconds, insert the pipette to a depth of 20 cm and withdraw exactly 20 ml. **This is the most important single step in this exercise as subsequent analyses are based on the calculation of the total mud weight.** Continue to withdraw 20-ml samples with the 20 ml volumetric pipette at the depths and times indicated on Table 1 for the recorded water temperature (from Lewis and McConchie,1994).

7.8.2 Transfer the pipette sample fractions to separate tared 50 ml glass beakers. Each pipette withdrawal should be rinsed with a small volume of deionized water which is then added to the 50 ml sample beaker.

7.8.3 Each beaker should be placed in an oven at 100^{o} C for 24 hrs. All weights are to be recorded on the data sheet.

7.8.4 Unused sediment from each sample will be stored at 4 to 5 °C for QA/QC analyses and other sediment analyses.

Table 1. Sampling Time Intervals for Pipette Analysis.

PHI DIAMETER	DIAMETER (microns)	PIPETTE SAMPLING DEPTH (cm)	Elasped time for withdrawal of sample in hours , minutes, and seconds (h:mm:ss). 18 degrees C	19 degrees C	20 degrees C	21 degrees C	22 degrees C	23 degrees C	24 degrees C	25 degrees C	26 degrees C
		Start Time:	0:00:00	0:00:00	0:00:00	0:00:00	0:00:00	0:00:00	0:00:00	0:00:00	0:00:00
4.0	62.5	20	0:00:20	0:00:20	0:00:20	0:00:20	0:00:20	0:00:20	0:00:20	0:00:20	0:00:20
4.5	44	20	0:02:02	0:01:59	0:01:56	0:01:53	0:01:50	0:01:48	0:01:45	0:01:43	0:01:40
		Restir until:	0:03:00	0:03:00	0:03:00	0:03:00	0:03:00	0:03:00	0:03:00	0:03:00	0:03:00
5.0	31	10	0:05:02	0:04:59	0:04:57	0:04:54	0:04:51	0:04:48	0:04:46	0:04:44	0:04:41
5.5	22	10	0:07:03	0:06:57	0:06:51	0:06:46	0:06:40	0:06:35	0:06:30	0:06:26	0:06:21
6.0	16	10	0:10:40	0:10:28	0:10:18	0:10:07	0:09:57	0:09:47	0:09:38	0:09:29	0:09:19
6.5	11	10	0:19:12	0:18:49	0:18:26	0:18:03	0:17:42	0:17:21	0:17:01	0:16:42	0:16:22
7.0	7.8	10	0:35:14	0:34:27	0:33:42	0:32:56	0:32:13	0:31:32	0:30:53	0:30:15	0:29:36
7.5	5.5	10	1:07:49	1:06:14	1:04:44	1:03:13	1:01:46	1:00:24	0:59:05	0:57:49	0:56:30
8.0	3.9	10	2:11:55	2:08:46	2:05:47	2:02:45	1:59:53	1:57:09	1:54:32	1:52:02	1:49:23
8.5	2.8	5	2:08:03	2:05:00	2:02:06	1:59:10	1:56:23	1:53:43	1:51:11	1:48:46	1:46:12
9.0	2.0	5	4:08:06	4:02:07	3:56:26	3:50:41	3:45:13	3:40:01	3:35:03	3:30:18	3:25:16

Table 1. continued. Calculations for determining sample withdrawal times for pipette analyses.

Template for Sediment Grain Size Analysis:

Sample withdrawal times for pipette analysis are based upon Stoke's law which can be written as:

$T = Depth/[1500*A*(d^2)]$ (Folk, 1968)

where T is time in minutes,

Depth is in centimeters,

A is a contant, and

d is the particle diameter in millimeters.

The A value is a function of temperature, gravity, and density of particles. We will assume a density of 2.65 (associated with quartz or clay minerals). The following table relates various temperatures to the A constant.

Temperature (degrees °C)	A
20	3.57
21	3.66
22	3.75
23	3.84
24	3.93
25	4.02
26	4.12

Temp ?		25	=>Enter temperature.
A value	=	4.02	=>The A value will automatically be determined.
Time?		0:00:00	=>Enter the start time for the analysis.

7.9 Treatment of the Sand Fraction (> 63 µm) Retained on the Sieve

7.9.1 Transfer the >63 μm fraction to a 250 ml glass beaker and place in a drying oven at 100°C until dry for 24 hrs.

7.9.2 Transfer the dried sediment into the top of a stack of clean, stainless steel sieves composed of 500 μm (1.0 Ø), 355 μm ($\approx$1.5 Ø), 250 μm (2.0 Ø), 180 μm ($\approx$2.5 Ø), and 125 μm (3.0 Ø), 90 μm (~3.5 Ø) and 63 μm (4.0 Ø) sieve with a closed pan on the bottom. Shake on a rotary tapper (Ro-tap) for 15 minutes.

7.9.3 Weigh each sieved fraction as follows: Tare a 100 ml beaker to zero; add the 500 μm (1.0 Ø) sediment fraction and weigh to 0.001 grams. Next add the 355 μm ($\approx$1.5 Ø) fraction to the beaker. Proceed to add subsequent fractions until all weighed. Record the individual and cumulative weights of the > 63 μm fraction to 0.001 grams.

7.10 Removal of Carbonates (if necessary):

7.10.1 If a sediment contains >50%, by weight, of calcareous material, the sample is described as a carbonate sediment (Blatt *et al*., 1975). The following steps will be followed to process carbonate sediments. Record the weight of the sand fraction to 0.001 grams. A 10% (by volume) HCl solution will be added to the dried and weighed sediment. Cover the sediment completely with HCl and let sit for four hours. Additional acid will be added and if foaming is apparent, the sample will be left to stand for several more hours. This process will be repeated until no further reaction occurs with subsequent additions of HCl.

7.10.2 The sample will be transferred to a 63 μm sieve and washed using copious amounts of deionized water. This will remove any salts formed during the acidification step.

7.10.3 The sample will be transferred to a 100 ml glass beaker, dried, and weighed to 0.001 grams. Proceed with steps in Section 7.9.

7.10.4 Calculate the sediment carbonate content:

$$\textbf{\% Carbonate} = \frac{\{\text{wt. of beaker and sed. (before acid) - wt. of beaker and sed. (after acid)}\}}{\{\text{ wt. of beaker and sed. (before acid) - beaker wt.}\}}$$

7.11 Calculations for Sediment Grain Size Distributions

7.11.1 Calculate the total weight of mud (silt-clay) in the sample (obtained @ 20 sec withdrawal).
Total mud weight (g) = [((beaker+ sed wt.) - beaker wt.) x 50] - dispersant weight

Dispersal weight = 0.1 gram, if procedures in Section 7.6.1 were followed.

(**note**: the amount of mud in each 20 ml withdrawal is equal to 1/50 of the total amount of mud remaining in the 1000 ml cylinder at the withdrawal time and at the withdrawal depth)

7.11.2 Calculate the total sample weight:

Total sample weight (g) = total mud weight + total sand weight

7.11.3 Determination of cumulative percentages for each mud (<63 µm)size fraction:

Each pipette sample represents material in the column finer than a certain grain size. To begin, multiply each size fraction by 50, subtract the weight of dispersant:

5Ø fraction wt. (g) = (5 Ø sample weight X 50) - dispersant weight (g)
6Ø fraction wt. (g) = (6 Ø sample weight-X 50) - dispersant weight (g)
7Ø fraction wt. (g) = (7 Ø sample weight X 50) - dispersant weight (g)
8Ø fraction wt. (g) = (8 Ø sample weight X 50) - dispersant weight (g)
9Ø fraction wt. (g) = (9 Ø sample weight X 50) - dispersant weight (g)

Then divide each fraction by the total sample weight, subtract from 1, and multiply the product by 100:

Cumulative % (at 5 Ø)= [1 - (5 Ø fraction wt. (g)/ Total sample weight (g))] x 100
Cumulative % (at 6 Ø)= [1 - (6 Ø fraction wt. (g)/ Total sample weight (g))] x 100
Cumulative % (at 7 Ø)= [1 - (7 Ø fraction wt. (g)/ Total sample weight (g))] x 100
Cumulative % (at 8 Ø)= [1 - (8 Ø fraction wt. (g)/ Total sample weight (g))] x 100
Cumulative % (at 9 Ø)= [1 - (9 Ø fraction wt. (g)/ Total sample weight (g))] x 100

7.11.4 Determination of cumulative percentages for each sand (<63 µm) fraction:

The weight of each sand fraction is:

% Wt. of each sand fraction = (wt. of sand for each Ø size / total sample weight) x 100

Add these percentages incrementally to obtain cumulative weight percentages.

7.12 Determination of Statistical Parameters of Grain Size:

7.12.1 Plot the cumulative curve of the sample and read the Ø values which correspond to the 24th ($Ø_{25}$), 50th (*i.e.*, median ($Md_{ø}$)) and 75th ($Ø_{75}$) percentiles by linear interpolation.

7.12.2 Calculate the Phi Quartile Deviation (QDØ):

Phi Quartile Deviation = $(Ø_{75} - Ø_{25}) / 2$

7.12.3 Calculate the Phi Quartile Skewness (SkqØ):

Phi Quartile Skewness = $((Ø_{25} + Ø_{75}) - (2 \times Md_{ø})) / 2$

7.12.4 Record the $Md_{ø}$, QDØ, and SkqØ for each sample.

8. PROCEDURES FOR SEDIMENT TOTAL ORGANIC CARBON DETERMINATION

8.1 The following procedures are used to determine the total organic carbon concentration in sediment. Total organic carbon will be determined by combusting pre-acidified samples at high temperature and measuring the volume of carbon dioxide gas produced (Salonen 1979b). Analytical procedures are generalized. Although the basic method used by various laboratories may be the same, instrumentation differences prohibit giving specific, step-by-step procedures.

8.2 Sample Preparation:

8.2.1 Sediment samples will be retrieved from cold storage and brought to room temperature. Sample numbers will be recorded on a data sheet upon retrieval from storage. Unlike other procedures, samples that have dried can be analyzed to determine total organic carbon concentration.

8.2.2 Sediments will be removed from storage bags, placed in a clean beaker, and homogenized. Homogenization will be accomplished by stirring sediment with a small metal spatula for at least three minutes.

8.2.3 A sample of at least 5 g wet weight (3.3 ml) will be placed in a tared evaporating dish and covered to protect the sample against contamination with carbon from other sources.

8.2.4 The sample will be placed in a drying oven at 60 °C until dry. Typically, 48 hours is sufficient for the dry weight of sediment samples to stabilize. A randomly selected subsample of each batch will be reweighed after an additional 24 hour drying period as a check for the stability of the dry weight measurement. All weights will be recorded on the data sheet.

8.2.5 The dried sediment will be transferred to a porcelain mortar and thoroughly ground with a porcelain pestle. Obvious large shell fragments should be removed with metal forceps, being careful not to contaminate the sample with foreign material.

8.2.6 The ground sediment will be placed in cleaned glass scintillation vials with foil-lined caps. All labels should remain on the exterior of the vials to minimize carbon contamination.

8.2.7 Samples prepared as above may be stored at room temperature indefinitely.

8.3 Sample Analysis:

8.3.1 Approximately 20 to 30 mg of the dried and ground sediment samples will be placed into small beakers. The samples will be acidified to remove sources of inorganic carbon (*e.g.*, shell fragments). This is accomplished by suspending the beakers over a concentrated pool of HCl in a sealed desiccator (Lambert and Oviatt, 1986). Samples are typically fumed for at least 15 hours.

8.3.2 After fuming, the samples are transferred to an oven and dried for an additional two hours (100°C). For muddy sediment, 5 to 10 mg of sample is placed inside an aluminum vial and crimpled into a small ball. For sandy sediment, as much as 50 to 100 mg of sample may be required due to the low organic content generally associated with sands.

8.3.3 For analyses, samples are typically placed in a tube furnace at approximately 950°C and exposed to a precombusted stream of oxygen. The CO_2 evolved is measured by an infrared gas analyzer, and the resulting gas peak is integrated. Integrator units are compared to a standard curve to convert to organic carbon.

8.3.4 Standard curves for analyses are typically made with a high purity organic compound such as D-glucose dissolved in low organic distilled water. Curves are checked with sediment standards (MESS and BCSS) from The Marine Analytical Chemistry Standards Program of the National Research Council of Canada.

9. QUALITY ASSURANCE AND QUALITY CONTROL PROCEDURES FOR SEDIMENT ANALYSES

9.1 Quality control for the sediment analysis procedures will be accomplished by reanalyzing samples that fail either a range check or recovery check. Quality assessment will include reanalysis of 10% of the samples. Quality assessment samples will be selected randomly. Reanalysis will consist of repeating the sediment analysis procedure on the archived sample collected from the same grab as the sample failing QC.

9.2 For the range check, any sample results that fall outside expected ranges will be reanalyzed. For example, any percentage that totals greater than 100% will be reanalyzed. For the recovery check, if the total weight of the recovered sands is 10% (by weight) less or greater than the starting weight of sands, the sample will be reanalyzed.

9.3 For Quality Assurance, samples will be selected randomly from each batch and reanalyzed. A batch of samples is a set of samples of a single textural classification *(i.e.*, mud), processed by a single technician, using a single procedure (*i.e.*, complete sediment analysis).

9.4 Sediment sample reanalysis will be done in the following manner:

9.4.1 Approximately 10% of each batch completed by the same technician will be reanalyzed.

9.4.2 A random selection of the samples will be processed in the same manner as the original sample batch.

9.4.3 If the absolute difference between the original number (silt-clay percentage, for example) and the second number is greater than 10% then a third analysis will be completed by a different technician.

9.4.4 The values closest to the third value will be entered into the data base.

9.4.5 If more than 10% of the data from a batch are in error, then the whole batch will be reprocessed using the archived sediment. A third check of the reanalyzed samples will be completed by a different technician to assure that the reanalyzed values are correct.

9.4.6 Reanalysis and QA checks must be accomplished within 30 days from the date the original sediment analysis was conducted.

9.4.7 Reanalysis and QA checks are dependent upon having enough sediment to complete the various reanalyses.

10. DATA FORMS

10.1 Blank copies of data forms used for the analysis of sediment samples are presented in Appendix A in the following order:

- Sediment data sheet for silt-clay analysis
- Sediment data sheet for grain-size distribution analysis
- Example probability paper for plotting grain-size distributions

11. REFERENCES

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APPENDIX A

DATA FORMS

(See SED-DATA worksheet in Sec5.xls)

(See GRAIN SIZE worksheet in Sec5.xls)

(See EXAMPLES worksheet in Sec5.xls)

(See EXAMPLES worksheet in Sec5.xls)

(See EXAMPLES worksheet in Sec5.xls)

(See EXAMPLES worksheet in Sec5.xls)

(See PIPETTE SHEET worksheet in Sec5.xls)

SECTION 6

RESIDUE, NON-FILTERABLE (SUSPENDED SOLIDS) (Gravimetric, Dried at 103-105°C)

1. SCOPE AND APPLICATION

1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes.

1.2 The practical range of the determination is 4 mg/L to 20,000 mg/L.

2. SUMMARY OF METHOD

2.1 A well-mixed sample is filtered through a glass fiber filter, and the residue retained on the filter is dried to constant weight at 103-105°C.

2.2 The filtrate from this method may be used for Residue, Filterable.

3. DEFINITIONS

3.1 Residue, non-filterable is defined as those solids which are retained by a glass fiber filter and dried to constant weight at 103-105°C.

4. SAMPLE HANDLING AND PRESERVATION

4.1 Non-representative particulates such as leaves, sticks, fish, and lumps of fecal matter should be excluded from the sample if it is determined that their inclusion is not desired in the final result.

4.2 Preservation of the sample is not practical; analysis should begin as soon as possible. Refrigeration or icing to 4°C, to minimize microbiological decomposition of solids, is recommended.

5. INTERFERENCES

5.1 Filtration apparatus, filter material, pre-washing, post-washing and drying temperature are specified because these variables have been shown to affect the results.

5.2 Samples high in Filterable Residue (dissolved solids), such as saline waters, brines and some wastes, may be subject to a positive interference. Care must be taken in selecting the filtering apparatus so that washing of the filter and any dissolved solids in the filter (7.5) minimizes this potential interference.

6. APPARATUS

6.1 Glass fiber filter discs, without organic binder, such as Millipore AP-40, Reeves Angel 934-AH, Gelman type A/E, or equivalent.

> **NOTE:** Because of the physical nature of glass fiber filters, the absolute pore size cannot be controlled or measured. Terms such as "pore size" collection efficiencies and effective retention are used to define this property in glass fiber filters. Values for these parameters vary for the filters listed above.

6.2 Filter support: Filtering apparatus with reservoir and a coarse (40-60 m) fritted disc as a filter support.

> **NOTE:** Many funnel designs are available in glass or porcelain. Some of the most common are Hirsch or Buchner funnels, membrane filter holders and Gooch crucibles. All are available with coarse fritted disc.

6.3 Suction flask.

6.4 Drying oven, 103-105°C.

6.5 Desiccator.

6.6 Analytical balance, capable of weighing to 0.1 mg.

7. PROCEDURE

7.1 Preparation of glass fiber filter disc: Place the glass fiber filter on the membrane filter apparatus or insert into bottom of suitable Gooch crucible with wrinkled surface up. While vacuum is applied, wash the disc with three successive 20 ml volumes of distilled water. Remove all traces of water by continuing to apply vacuum after water has passed through. Remove filter from membrane filter apparatus or both crucible and filter if Gooch crucible is used, and dry in an oven at 103-105°C for one hour. Remove to desiccator and store until needed. Repeat the drying cycle until a constant weight is obtained (weight loss is less than 0.5 mg). Weigh immediately before use. After weighing, hand the filter or crucible/filter with forceps or tongs only.

7.2 Selection of Sample Volume

> For a 4.7 cm diameter filter, filter 100 mL of sample. If weight of captured residue is less than 1.0 mg, the sample volume must be increased to provide at least 1.0 mg of residue. If other filter diameters are used, start with a sample volume equal to 7 ml/cm^2 of filter area and collect at least a weight of residue proportional to the 1.0 mg stated above.

NOTE: If during filtration of this initial volume the filtration rate drops rapidly, or if filtration time exceeds five to ten min, the following scheme is recommended: Use an unweighed glass fiber filter of choice affixed in the filter assembly. Add a known volume of sample to the filter funnel and record the time elapsed after selected volumes have passed through the filter. Twenty-five ml increments for timing are suggested. Continue to record the time and volume in increments until filtration rate drops rapidly. Add additional sample if the filter funnel volume is inadequate to reach a reduced rate. Plot the observed time versus volume filtered. Select the proper filtration volume as that just short of the time a significant change in filtration rate occurred.

7.3 Assemble the filtering apparatus and begin suction. Wet the filter with a small volume of distilled water to seat it against the fritted support.

7.4 Shake the sample vigorously and quantitatively transfer the predetermined sample volume selected in Sect. 7.2 to the filter using a graduated cylinder. Remove all traces of water by continuing to apply vacuum after sample has passed through.

7.5 With suction on, wash the graduated cylinder, filter, non-filterable residue and filter funnel wall with three portions of distilled water allowing complete drainage between washing. Remove all traces of water by continuing to apply vacuum after water has passed through.

NOTE: Total volume of wash water used should equal approximately 2 ml/cm^2. For a 4.7 cm filter the total volume is 30 mL.

7.6 Carefully remove the filter from the filter support. Alternatively, remove crucible and filter from crucible adapter. Dry at least one hour at 103-105°C. Cool in a desiccator and weigh. Repeat the drying cycle until a constant weight is obtained (weight loss is less than 0.5 mg).

8. CALCULATIONS

8.1 Calculate non-filterable residue as follows:

$$Non\text{-}filterable\ residue\ (mg/L) = \frac{(A-B)\ x\ 1{,}000}{C}$$

where:

A = weight of filter (or filter and crucible) + residue in mg
B = weight of filter (or filter and crucible) in mg
C = ml of sample filtered

9. PRECISION AND ACCURACY

9.1 Precision data not available at this time.

9.2 Accuracy data on actual sample cannot be obtained.

10. REFERENCE

NCASI Technical Bulletin No. 291, March 1977. National Council of the Paper Industry for Air and Stream Improvement, Inc., 260 Madison Ave., New York.