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**FINAL
WORK/QUALITY ASSURANCE PROJECT PLAN**

**NARRAGANSETT BAY ECORISK AND MONITORING
FOR NAVY SITES**

Prepared for:

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LIST OF ACRONYMS

<u>ACRONYM</u>	<u>DESCRIPTION</u>
ACOE	Army Corps of Engineers
AVS	Acid Volatile Sulfide
AWQC	Ambient Water Quality Criteria
BNA	Base/Neutrals and Acids
BSAF	Biota to Sediment Accumulation Factor
COC	Contaminants of Concern
CS	Confirmation Study
4,4'-DDD	1,1-Dichloro -2,2-bis (p-chlorophenyl) ethane
ECRA	Environmental Clean-up Responsibility Act
EMAP	Environmental Monitoring and Assessment Program
ERA	Ecological Risk Assessment
ERL	Effects Range Low
ERM	Effects Range Median
EPA	Environmental Protection Agency
FFA	Federal Facility Agreement
HQ	Hazard Quotient
IAS	Initial Assessment Study
LOAEL	Lowest Observed Adverse Effect Level
NEESA	Naval Energy and Environmental Support Activity
NETC	Naval Education and Training Center
NOAA	National Oceanic and Atmospheric Administration
NPL	National Priorities List
NUSC	Naval Underwater Systems Center
PAH	Polynuclear Aromatic Hydrocarbon
PCB	Polychlorinated Biphenyl
QA/QC	Quality Assessment/Quality Control
RI	Remedial Investigation
RIDEM	Rhode Island Department of Environmental Management
RI/FS	Remedial Investigation/Feasibility Study
ROD	Record of Decision
SAIC	Science Applications International Corporation
SQC	Sediment Quality Criteria
SVOC	Semi-Volatile Organic Compound
TAL	Target Analyte List
TBT	Tributyltin
TOC	Total Organic Carbon
TPH	Total Petroleum Hydrocarbon
TRC	TRC Environmental Corporation
URI	University of Rhode Island
VOC	Volatile Organic Compound

1.0 PROJECT DESCRIPTION

1.1 INTRODUCTION

The U.S. Navy has a long history in Narragansett Bay, Rhode Island. Primary installations and fortifications occur on Aquidneck, Conanicut, and Prudence Islands, as well as on the western side of the bay at Davisville and Quonset Point (Figure 1-1). Several of these facilities currently have requirements under the Comprehensive Environmental Resource Compensation and Liability Act (CERCLA), the National Contingency Plan (NCP), and Rhode Island State Statutes to assess the risks of hazardous waste disposal sites on human health and the environment. These requirements include evaluation of the risks posed by land-based hazardous waste sites to the ecology of Narragansett Bay.

The purpose of work identified in this Work Plan is to develop the information needed to prepare site-specific baseline ecological risk (ecorisk) assessments for Navy sites located in Narragansett Bay. The activities described in this Work Plan will provide the data necessary to complete ecological risk assessments for hazardous waste disposal sites located at the Naval Educational and Training Center Newport (NETC) and Naval Construction Battalion Center Davisville-Disestablished (NCBC) in Narragansett Bay, Rhode Island. The procedures and data collection activities identified in this Work Plan have been developed to satisfy the ecorisk requirements for several sites located on Narragansett Bay, RI. These sites include McAllister Point Landfill (NETC Newport), Old Fire Fighting Training Area (NETC Newport), Derecktor Shipyard/Coddington Cove (NETC Newport), Melville North Landfill (NETC Newport), and Allen Harbor (NCBC Davisville). The Work Plan identifies a consistent approach for the development of assessment and measurement endpoints required to assess ecological risks in Narragansett Bay and will incorporate specific concerns identified by the Narragansett Bay Ecorisk Advisory Group.

The Work/Quality Assurance Project Plan ("Work Plan") for Narragansett Bay Ecorisk and Monitoring for Navy Sites is intended to be a master Work Plan placing all Navy ecological risk assessment and monitoring activities in Narragansett Bay into a single comprehensive context. This document provides generic background information concerning approaches to Problem Formulation, Exposure and Ecological Effects Assessments, Risk Characterization, sampling and analysis methodologies, and QA/QC requirements and activities, so that this material need not to be recreated for each site-specific investigation. Site-specific ecological risk assessment activities, including sampling and analysis plans specific to individual Navy sites, are attached as addenda. Each site-specific addenda includes descriptions of existing data and gaps that exist for that site, as well as the activities planned to fill those gaps, and will be submitted for regulatory review prior to initiation of activities at each site.

1.2 BACKGROUND

The Naval facilities NETC Newport and NCBC Davisville are located in the southern and central portions of Narragansett Bay, respectively (Figure 1-1). Both are listed on the National Priorities List (NPL), and have signed Federal Facility Agreements in place. NCBC is being closed under Base Reutilization and Closure (BRAC) procedures. Both NETC and NCBC have requirements under the Comprehensive Environmental Resource Compensation and Liability Act (CERCLA), the National Contingency Plan (NCP), and Rhode Island State Statutes to assess the risks of hazardous waste disposal sites on human health and the environment, and to identify appropriate cleanup levels (Johnston and Nixon 1994). There is an extensive amount of data and information on Narragansett Bay which has been generated by the Graduate School of Oceanography (GSO) of the University of Rhode Island (URI), URI's Coastal Resource Center, the U.S. Environmental Protection Agency's (U.S. EPA) Narragansett Bay Project, the U.S. EPA's Environmental Research Laboratory at Narragansett (ERL-Narragansett), and the National Oceanic and Atmospheric Administration's (NOAA) Coastal Ocean Program. This information will be used, as appropriate, in the ecological risk assessments and will provide a context for interpreting the results of site-specific studies.

Currently available monitoring data have indicated that there is a general north-to-south gradient of organic and heavy metal contamination in the waters and sediments of Narragansett Bay (Figure 1-2) (Hurt and Quinn 1979; Pruell 1984; Pruell and Quinn 1985; Corbin 1989; Quinn *et al.* 1992; King *et al.* in prep), with highest concentrations measured in the Providence River to the north.

Project tasks described in this work plan have been developed so that, upon their completion, new information would close data gaps and support the completion of ecological risk assessments. This information will be used by the Navy to satisfy specific regulatory requirements.

1.3 OBJECTIVES, SCOPE, AND ORGANIZATION OF MASTER PLAN

Based on U.S. EPA's general and Region 1 guidance for ecological risk assessment, and using data and information for Narragansett Bay, the overall goal of this Work Plan is to identify and describe activities needed to generate and interpret the data required to complete ecological risk assessments for Navy disposal sites in Narragansett Bay. The project will also be prepared to address specific concerns identified by the Narragansett Bay Ecorisk Advisory Group, as they arise.

Through examination of the relationship between contaminant exposure and effects levels, the overall ecological impact of Navy-related activities in the vicinity of Navy disposal areas in Narragansett Bay will be discerned. The exposure, effects and

risks associated with particular sites will be generated through activities described in the Work Plan as well as site specific addenda attached to the Work Plan.

The primary objectives of the ecological risk assessments conducted under this Work Plan and the addenda are threefold:

1. To assess the ecological risks to offshore environments of Narragansett Bay from chemical stressors associated with individual land-based hazardous waste sites at NETC Newport and NCBC Davisville.
2. To develop information sufficient to make informed risk management decisions regarding remedial options on a site-specific basis.
3. To support communication of the nature and extent of ecological risks associated with Navy sites to the public.

To guarantee the required activities are conducted to meet these objectives, this Work Plan was developed following general guidance provided by the U.S. EPA (1989, 1992, 1994) and incorporates input provided by U.S. EPA Region I, the State of Rhode Island, and Natural Resource Trustees, representatives of which jointly constitute the Narragansett Bay Ecorisk Advisory Group (see Addenda for identification of specific group members). The scope of the ERA's conducted under this Work Plan include:

1. Problem Formulation and the determination of the nature and extent of contamination of offshore media associated with Navy sources. Specifically, this activity involves identification of contaminated media, identification of contaminants of concern (CoCs), evaluation of the spatial extent of contamination, identification of the ecological receptors potentially at risk from CoCs, and identification of appropriate assessment and measurement endpoints. The information generated during the Problem Formulation is integrated into a conceptual model, which identifies the possible exposure scenarios and mechanisms of ecological impact associated with the CoCs.
2. Exposure and Ecological Effects Assessments, involving the collection of information needed to quantify chemical exposures and the observed or predicted ecological effects resulting from exposure. Exposure Assessment involves quantification or estimation of the concentrations of CoCs in environmental media in the exposure pathways from source to ecological receptors. Ecological Effects Assessment involves a combination of toxicological literature review, *in situ* characterizations of the status of receptor species, measures of the toxicity of exposure media, and modeling exercises used to predict the occurrence of adverse ecological impact. Site-specific Exposure and Ecological Effects Assessment activities are

determined by the conceptual model developed at the end of Problem Formulation.

3. Characterization of Ecological Risks utilizing a weight of evidence approach involving comparisons of observed adverse effects with conditions at reference stations, analysis of CoC concentration *versus* observations of adverse effects, analysis of CoC bioaccumulation, comparisons of toxicity evaluations with observed ecological effects, comparisons of exposure point concentrations with established standards and criteria for offshore media, and comparisons of exposure point concentrations with published information regarding the toxicity of CoCs. The results of these analyses will be summarized together with all information obtained during each study to characterize ecological risks associated with each Navy site in Narragansett Bay.
4. Communication of the study objectives, methods, and findings of the ecological risk assessment in a format which supports informed risk management decisions for each site.

The format of this draft final Work Plan follows guidance provided by the Narragansett Bay Ecorisk Advisory Group and incorporates comments received on the draft version of this document. Section 2.0 of the Work Plan provides a general plan for ecological risk assessments to be conducted at Navy sites in Narragansett Bay requiring regulatory attention, and includes the presentation of the approaches to be followed for completion of Problem Formulation, Exposure and Ecological Effects Assessments, and Risk Characterization activities at each site. To aid in site-specific Problem Formulation activities, a summary of site characterization information and a generalized conceptual model are presented in Section 2.1. Program-wide data quality requirements and sample collection and analysis procedures are presented in Section 3.0. Analytical procedures proposed for use throughout the program are described in Section 4.0. These procedures are based upon the data requirements imposed by ecological risk assessments conducted in the marine environment. Section 5.0 describes sample and data management procedures to be employed in each investigation. General descriptions of the site-specific ecological risk assessment reports produced for each investigation, as well as identification of the data products and how they will be used to support the ecological risk assessment for each site, are given in Section 6.0 of the master Work Plan. The Health and Safety Plan and approaches for ensuring that risks to project personnel will be minimized are presented in Section 7.0. Section 8.0 includes a list of all references cited of all references cited in the master Work Plan. Appendices are attached which include Standard Operating Procedures (A), QA/QC protocols unique to analytical chemistry and toxicity (B), the program Health and Safety Plan (C).

Building upon the foundation provided in the master Work Plan, site-specific plans for ecological risk assessments are attached as addenda to this document. These plans describe the proposed application of approaches described in Sections 2.0-7.0 to each site-specific investigation. Each addendum will present the results of Problem Formulation for that site, will identify existing data gaps and approaches to obtaining the necessary data (Field Sampling and Analysis Plans), and will describe Exposure Assessment, Ecological Effects Assessment, and Risk Characterization activities unique to each site. Whereas investigations conducted at individual sites at NETC Newport and NCBC Davisville will be conducted by different teams of project personnel, project organization and responsibilities also are described in each addendum.

2.0 GENERAL PLAN FOR ECOLOGICAL RISK ASSESSMENTS

The research and monitoring strategy developed for the marine ecological risk assessment for Navy Sites in middle and southern Narragansett Bay builds upon techniques and methods applied for marine and estuarine ecological risk assessment case studies performed at NCBC Davisville, in Narragansett Bay, Rhode Island, and the Naval Shipyard Portsmouth, Kittery, Maine. The original pilot study in Narragansett Bay provided significant information and experience in assessing ecological risks to marine systems from past hazardous waste disposal practices (NOSC and ERLN 1990; Johnston *et al.* 1990; Munns *et al.* 1991, 1992, 1994a; Mueller *et al.* 1992; Johnston and Nixon 1994). Improvements and refinements of methods for assessing ecological risks were incorporated into the strategy employed in the study being conducted for the Naval Shipyard Portsmouth. This experience has helped to define the information needed in marine ecological risk assessments for application to the lower Narragansett Bay in support of the Remedial Investigation/Feasibility Studies (RI/FS) for NETC Newport and NCBC Davisville.

This project will be implemented following guidance provided by the U.S. EPA Risk Assessment Forum's "Framework for Ecological Risk Assessment" (U.S. EPA 1992; Norton *et al.* 1992), with additional guidance provided by U.S. EPA (1989, 1994) and input from the Narragansett Bay Ecorisk Advisory Group. The framework is intended to provide a logical overarching structure for conducting risk assessments, and to enhance uniformity among assessments. This latter intent is particularly important to decision makers who must evaluate risks associated with various management options, perhaps as estimated by different assessors. The framework is intended to be general with respect to the nature of the stressor(s) and the ecological systems involved in any given assessment. It therefore has utility in assessments involving both chemical and nonchemical stressors, and all types of ecological systems.

The U.S. EPA Framework document itself consists of three major components; *Problem Formulation*, *Analysis*, and *Risk Characterization* (Figure 2-1). During the

first of these, *Problem Formulation*, planning and scoping activities are directed towards delineation of the overall goals, activities, and boundaries of the assessment. Consideration is given to the characteristics of the stressors, observed or suspected ecological effects, and the ecological systems potentially at risk. This information is synthesized into a conceptual model which summarizes understanding of the problem as a series of working hypotheses. The *Analysis* component consists of exposure and ecological effects assessments. In the exposure assessment, the transport and transformation of contaminants and their contact with endpoint species (receptors) are evaluated on a contaminant by contaminant basis. Exposure pathways are identified and quantified by characterizing the nature and extent (spatial distribution) of the contamination by a combination of data collection, sample manipulation, and modeling exercises. This information, coupled with the available life history data for the receptors is used to define the magnitude of exposure in time and space. In the ecological effects assessment, toxicity evaluations taken together with field observations of adverse effects are used to characterize the relationships between possible ecological impacts and stressor exposure. In *Risk Characterization*, the relationship between contaminant exposure and ecological effects are defined by a weight-of-evidence approach (e.g., triad approach) which includes ecological survey data, chemistry data, and toxicity data derived from laboratory bioassays. Potential confounding effects related to site-specific physical/biological influences not related to chemical contaminants are also considered. Detailed exploration of relationships between contaminant exposure and observed effects will also be done as part of the risk characterization. Other approaches, such as joint probability of exposure and effects and/or simulation modeling of contaminant exposure pathways and effects scenarios (e.g. population models) may also be used for site-specific risk characterizations. Assessment results will be communicated in a format useful to the risk manager, with an evaluation of the uncertainties associated with assessment conclusions. Finally, environmental monitoring is required to validate the risk assessment and to verify the effectiveness of remediation alternatives.

2.1 APPROACH FOR PROBLEM FORMULATION

The following material addresses each of the components of Problem Formulation with respect to the offshore ecological risk assessment for lower Narragansett Bay (Figure 2-2). Gaps in the information required to conduct the assessment are identified where appropriate. A general site characterization provides a synopsis of existing data for Narragansett Bay to define additional data requirements for the marine ecological risk assessment. Next the plan for selection of contaminants of concern at each Navy site is developed, as is the plan for identifying ecological systems/species/receptors of concern. The final step of problem formulation involves the development of a conceptual model of ecological risks at each site. Approaches to each of these tasks are provided below.

2.1.1 General Site Characterization for the Lower Narragansett Bay

2.1.1.1 General Setting

The Narragansett Bay System (NBS), located in southeastern New England, trends north-south. Glacial processes modified a fluvial drainage system, filling the bedrock valleys with sediment. As sea level inundated the glacial sediment surface, creating Narragansett Bay, paralic and estuarine sediments were deposited (McMaster 1984; Peck 1989). Presently, sediment accumulation in the NBS is governed by estuarine sedimentation processes that have deposited fine sediment in the sheltered upper and middle bay and more sandy sediment toward the mouths of the passages (McMaster 1960). Nichols (1986) has estimated that Narragansett Bay can readily accommodate all the sediment input it receives.

The NBS covers an area of 328 km², draining 4708 km², most of which lies in Massachusetts (Olsen *et al.* 1980; Pilson 1985). The lower two-thirds of the NBS is characteristic of a well mixed estuary; although the Providence River becomes partially mixed due to the freshwater input (Olsen *et al.* 1980). Rivers emptying into the Providence River and Mt. Hope Bay account for almost 90 percent of the fresh water input to the NBS and drain both urban and rural areas (Olsen *et al.* 1980). Tides are semidiurnal and range from 1.1 m at the mouth of the NBS to 1.4 m at the head (Chinman and Nixon 1985). The non-tidal surface current pattern for the NBS is shown in Figure 2-3 (Hicks 1959; McMaster 1960).

Narragansett Bay is a small to medium sized estuary compared to other estuaries in the United States; however, the bay, as a resource, has a major influence on many aspects of Rhode Island's economy. One such influence has been the presence of U.S. Navy activity in the lower east passage of the bay at NETC. NETC has been an active Naval facility since 1869, and currently extends along the western shore of Aquidneck Island within portions of Newport, Middletown and Portsmouth, RI. Activities at NETC have included fueling of destroyers and cruisers, torpedo development, and training of Navy personnel (U.S. DHHS 1993).

2.1.1.2 Physical Oceanography

Narragansett Bay is a temperate, partially to well mixed estuary. The strong mixing is reflected by the fact that NBS water exhibits a nearly spatially uniform reduction in salinity relative to the more saline shelf water. There is no evidence for a direct fresh water jet through which fresh water is vented directly to the shelf (Pilson 1985). The Bay is well mixed because of strong tidal motions interacting with highly variable bottom topography. Mixing is more complete than other east coast estuaries such as Chesapeake Bay and the Patuxent River because of the larger tidal range of 55-60 cm in the Bay, compared to only 30 cm in Chesapeake Bay. Maximum vertical salinity gradients are limited to 2-3%. The estuarine system is composed of three

distinct north-south branches including the East and West Passages of the Bay and the Sakonnet River. The Providence and Taunton Rivers provide a source of fresh water inflow from the north and the Bay is bounded to the south by the salty inner shelf water of Rhode Island Sound (RIS). Fresh water discharge varies between a minimum 20 m³/s in late summer-fall to >300 m³/s under peak runoff conditions during winter-spring months. NBS is relatively shallow with estimates of mean depth ranging from 7.6 to 10 meters. Maximum depths occur in the East Passage where mean and maximum water depths are 18 and 24 meters, respectively.

Circulation in the Bay is driven by competing tidal, wind and density forcing. As outlined above, Weisburg (1976) showed that in the upper Bay wind and tidal forcing are of roughly equal importance. His results indicated the upper Bay was dominated by local wind events. Gordan and Spaulding (1987), in a study of the interaction between wind and tidal flow in the Bay, suggest from sea level measurements that non-local wind events may be important in the lower reaches of the Bay. Although, density stratification is weak (2%) in the Bay, mean flow patterns exhibit characteristics of classical 2-layer circulation (Hicks 1963) and, therefore, gravitational circulation must be considered when modeling long term transport properties within the Bay and between the Bay and RIS. As pointed out by Blumberg (1978), resolution of the mean density driven, baroclinic flow and how this flow interacts with wind driven flow, is essential for studies of transport of chemical and/or biological tracers.

2.1.1.3 Sediments of the Narragansett Bay System

The general surface sediment distribution of Narragansett Bay and adjacent RIS (Figure 2-4) was determined by a study of samples from 942 stations by McMaster (1960). This study found the subtidal sediments in the vicinity of the NETC study area to be silty sand. However, data were not obtained to characterize sediments within the coves and depositional areas along the west coast of Aquidneck Island.

A more recent study (French *et al.* 1992a) determined the relationship between benthic organisms and sediment type (primarily derived from McMaster, 1960), and a generalized map of sub-tidal benthic habitat types (Figure 2-5) was produced for lower Narragansett Bay and adjacent RI Sound. These habitats are described in Table 2-1. The two dominant habitats in the Lower East Passage are: 1) Lower Bay Complex - "a lower bay complex on a variety of mixed sediments containing sand, *Mytilus* (mussel) and *Crepidula* (slipper shells) shells are locally abundant. The mid-estuarine and estuarine-offshore species found in this habitat are *Pherusa affinis* (deposit feeding polychaetes), *Aricidea* (polychaetes), and *Ampelisca vaderum* (amphipod crustacean)" (French *et al.* 1992); and 2) Marine Silty Sand - this habitat is "a silty sand habitat typical of Rhode Island Sound and extending up into the East Passage. The [benthic] fauna are characterized by marine species such as *Astarte* (bivalve), *Cyclocardium* (bivalve), *Byblis serrata* (amphipod), and *Arctica islandica* (bivalve)"

(French *et al.* 1992a). The habitat classification did not extend into the coves and depositional areas located in the Lower East Passage study area. The classification of sediments not evaluated by McMaster (1960) and French *et al.* (1992a) in these coves represents a data collection activity to be addressed by this project. A combination of the results obtained by the side-scan and sub-bottom sonar studies and the chemical and lithologic studies of surface sediments and cores will allow us to produce a detailed distribution map of sediment types and sediment contamination levels in the study area. This information will allow us to assess the degree and extent of contamination in the study area and the potential for transport of the contamination.

2.1.1.4 Biological Studies of the Study Area.

Benthic Biology. Information available from the study area concerning the biology of benthic communities is quite limited. The only recent quantitative study of benthos in the Lower East Passage study area was conducted off Coasters Harbor (Metcalf and Eddy 1985; French *et al.* 1992b). Samples were taken at eleven stations on three dates from sediment types ranging from sand to silty sand (Metcalf and Eddy 1985). The amphipods *Leptocheirus pinguis* and *Casco bigelowii* were the most abundant species in many samples. The burrowing activities of these species create a soft, high-water content and well-oxygenated sedimentary environment (French *et al.* 1992b). It is likely that these activities also exert an important control on geochemical cycling between the sediment and the water column in areas where amphipods are dominant.

Shellfishing is of limited importance in the Lower East Passage study area. The vast majority of the study area is closed to shellfishing, and hard shell clam (*Mercenaria mercenaria*) fishing is done only adjacent to the closure line (Figure 2-6, from Pratt *et al.* 1992). *Mercenaria mercenaria* is commonly found in the coves of the Lower East Passage. A viable fishery is theoretically possible if the closure area is modified in the future. Lobster fishing is an important activity within the Lower East Passage. However, the information available on the distribution of lobsters is limited. The best information presently available was obtained by French *et al.* (1992c) by studying the distribution of lobster trap buoys. An example is shown in Figure 2-7 (French *et al.* 1992c). Obviously, areas in the Lower East Passage are among the most intensively fished in Narragansett Bay.

Other Organisms. The distributions in the NETC study area of other important organisms are mapped in French *et al.* (1992a, b, c). These organisms include eelgrass (*Zostera*) and macroalgae, finfish, and plants, amphibians, reptiles, birds, and mammals. Critical habitats for plants and birds exist within the Lower East Passage study area. In addition, well developed eelgrass and macroalgae habitats exist within the study area.

2.1.1.5 Chemistry Studies of Study Area

A limited number of investigations over the past 18 years have reported concentrations of organic and metal contaminants in the general area of the NETC. The analytes, methods and QA/QC procedures in these studies have varied widely and, therefore, some of the data may be suspect. However, several of the more recent investigations have used up-to-date analytical techniques and strong QA/QC protocols. Sediment cores were collected from several stations in the NETC area in 1976-1977 and analyzed for petroleum hydrocarbons (PHCs) (Hurtt and Quinn 1979). This work was part of a larger study whereby cores from 20 different locations were sampled and analyzed to describe the distribution of PHCs throughout Narragansett Bay. In general, there was a decrease in surface (0-5 cm) sediment hydrocarbons from the Providence River to the mouth of the bay in both passages (509 ppm to 35 ppm), and the concentrations also decreased with depth in the cores, generally leveling off at 20-25 cm. This depth is probably related to increased petroleum utilization starting at the end of the 19th century and increasing up to the present time. Several areas of the bay showed increasing PHCs with depth, especially four stations that were collected from the upper east passage between Melville and Hog Island. The actual source(s) of these hydrocarbons could not be determined, but the sources were probably active between 1940 to 1960 based on the estimated sedimentation rates at these locations. In addition, a core collected southeast of Prudence Island (Prudence Island Dumping Ground) also had increasing PHCs with depth, although these hydrocarbons probably came from dredged material that was dumped at this location.

In 1984, Energy Resources Company, Inc. reported on the analysis of polychlorinated biphenyls (PCBs) in surface sediments from an area north of Gould Island to south of Coasters Harbor Island, including the Newport Sewage Treatment Plant (STP) outfall off Coddington Point (ERCO 1984). These values ranged from ND (<0.1 ppm) to 0.28 ppm at a station near the outfall. The report also includes the GC/MS analyses of many other organic pollutants, but all values were below the detection limit of about 5 ppm. In a related study, Metcalf and Eddy, Inc. (1985) reported on the concentration of trace metals, organics, nutrients suspended solids, etc. in the Newport STP effluent. The data showed that all toxic pollutants were at or below the detection limits (DLs) with the exception of total phenolics and copper; however, many of the DLs were relatively high by today's standards.

Sediments (top 10-20 cm) and mussel (*Mytilus edulis*) samples were collected from Gould Island and McAllister Point and analyzed for total PCBs, PHCs and six trace metals (Cd, Cr, Cu, Ni, Pb and Zn) in 1988 (U.S. Army Corps of Engineers 1988). No details on analytical procedures or QA/QC protocols were included in this report. The range of reported values were as shown in Table 2-2 for PCBs, PHCs, and the relatively toxic metal, cadmium (Cd).

As part of a multidisciplinary investigation on the sediments of the bay in 1987-1989, sediment trap material, surface sediments (0-1 cm), hard shell clams (*Mercenaria mercenaria*), and sediment cores from 21 stations throughout the bay were analyzed for organic and metal contaminants for the Narragansett Bay Project (Quinn *et al.* 1992). Several of the stations for surface sediment were in the NETC area ranging from south of Hog Island (Station 12) to Fort Wetherill (Station 16), and the values for PHCs, PCBs and polycyclic aromatic hydrocarbons (PAHs) at these stations are shown in Table 2-3. In general there was a trend of decreasing concentration for all components from station 12 to station 16 with the exception of Newport Harbor (Station 15) which had elevated values for all three contaminant groups. Possible sources of these components include sewage effluent, combined sewer overflow (CSO) and industrial/military discharges, runoff and boating activities in the harbor area.

In 1993, Battelle Ocean Sciences was contracted by TRC Environmental Corporation to conduct field collection of sediments and bivalves, and laboratory analyses of samples associated with an off-shore investigation of NETC. The samples were collected in August 1993 and the report was submitted in February 1994 (Battelle Ocean Sciences 1994). Of the three sites examined, McAllister Point showed the highest levels of contamination. Significantly elevated concentrations of PCBs were measured in the sediments (0-15 cm) along a segment of the shore, with the highest levels (582 ppb) measured for a composite sample from 3 stations located close to the apex of the point. PCBs in mussels collected at the point were significantly elevated, but no notable elevation was observed for hard shell clams. The concentrations of most metals were also elevated in the sediment composite sample; and silver, lead and mercury were elevated in mussels and clams. PAHs were slightly elevated in several stations from the southern side of the point (~5 ppm).

The Old Fire Fighting Training area had high levels of pyrogenic PAHs in the sediments, mussels, and clams along a segment of the shore in Coasters Harbor. PCBs and metals, however, were not significantly elevated at the location. The third site, Melville North Landfill, was least impacted by pollutants; there was no significant contamination at this site.

In the most recent examination of sediment chemistry associated with NETC, scientists from the Graduate School of Oceanography, University of Rhode Island (GSO/URI), conducted during November 1993 and June 1994, chemical contaminants in sediments from the former Derecktor Shipyard site at Coddington Cove were investigated. The results of this study were reported by Quinn *et al.* (1994):

1. The levels of organic components and trace metals from primarily anthropogenic origin are very high in Coddington Cove surface sediments relative to concentrations typical of lower Narragansett Bay sediments. In addition, the concentrations of the Σ PCBs, Σ PAHs, and pp' DDE found at a

few of the cove stations are similar to or exceed values found in the Providence River. The concentrations of nickel and zinc exceed those typically observed in the Providence River and the levels of copper and lead are comparable to those observed at this location.

2. Elevated concentrations of the Σ PCBs and Σ PAHs were found in the one clam sample of sufficient size for analysis. These values are similar to those in clams from the upper Narragansett Bay and the Providence River.
3. The spatial distribution of organic contaminants and trace metals in the surface sediments of Coddington Cove, after normalization for lithologic variations (Aluminum normalization), indicates that the primary sources for many of these components are the series of outfall pipes from the former Derecktor Shipyard (e.g. stations 1 to 4) and/or piers for shipping activity (e.g. stations 11 and 20). Normalized concentrations are highest in the proximity of the outfall pipes and decrease with distance away from the outfalls.
4. The maximum concentrations of Σ PCBs, Σ PAHs, nickel, lead, and zinc observed in Coddington Cove sediments exceed the NOAA Effects Range-Medium (ER-M) guidelines (Long et al., 1995). These results indicate the potential exists for biological effects from exposure to organic and metal contaminants in the sediments of Coddington Cove.
5. The acid volatile sulfide (AVS) concentrations observed in Coddington Cove sediments are relatively high and are significantly higher than the sum of the concentrations of the simultaneously extracted metals (SEM). Therefore, the observed ratios of SEM/AVS are significantly lower than 1.0. The results of the AVS and SEM studies indicated that the potential for biological effects from exposure to trace metals in the sediments of Coddington Cove is significantly lower under present conditions than would be predicted by comparison of the concentration data with NOAA guidelines.
6. High concentrations of organic and trace metal contaminants are found in sediment core sections down to 31 cm depth. Based on an estimated sedimentation rate of about 1 cm/yr., the elevated contaminant levels could extend down to 50-60 cm in depth. In some cases, subsurface maxima in concentrations suggest higher anthropogenic inputs to the cove in the past relative to the present time.
7. Analysis of surface sediments from the mouth of Coddington Cove indicates that this area is not affected by organic contaminants from the cove or from the adjacent Newport Sewage Treatment Plant outfall at Coddington Point.

In addition to this general background, site characterizations specific to individual sites investigated as part of this program will be developed using information and data available at the time of site-specific Addendum development. These characterizations will aid in the understanding of site conditions, identification of Contaminants of Concern (CoCs), ecological systems/species/receptors of concern, and potential exposure pathways, and will support development of site-specific conceptual models of ecological risk.

2.1.2 Plan for Selection of Assessment and Measurement Endpoints of Concern, Including Contaminants and Species

2.1.2.1 Selection of Contaminants of Concern

The Narragansett Bay Ecorisk and Monitoring for Navy Sites program was initiated in response to the regulatory requirements of the RI/FS for NETC Newport and NCBC Davisville, through recognition of a number of potential stressors associated with past disposal practices and other Naval operations. These include chemical contaminants potentially linked with past activities at each facility. Based upon information obtained during previous sampling efforts (e.g., Quinn *et al.* 1994; Battelle Ocean Sciences 1994) and on-shore investigations (e.g., TRC Environmental Corporation, Inc. 1994), the list of chemical stressors includes both metals (Hg, Ni, Zn, Cu, Cd, Cr and Pb) and organic compounds (PAHs, PCBs, butyltins, and organochlorine pesticides (OCPs)). In response to the regulatory requirements of CERCLA and with an understanding of their toxicological importance and persistence in estuarine systems, these chemicals are considered as the primary stressors of concern in site-specific assessments.

Proposed Contaminants of Concern will be identified for each site using a rationale which links the source (e.g., McAllister Point Landfill) to potential marine receptors in Narragansett Bay through plausible exposure pathways. The process for their identification will involve a four-step process:

1. Using a hazard quotient (HQ) approach, chemical concentrations in site exposure media (such as ground water) will be compared with appropriately conservative biological benchmarks for these media (such as ambient water quality criteria) to identify contaminants elevated above levels presumed to be protective of biological systems. Chemicals which exceed (or nearly exceed, e.g. > 0.7) the biological benchmark will be termed "preliminary source CoCs".
2. Similarly, chemical concentrations in marine exposure media, including sediments and pore waters will be compared with appropriately conservative biological benchmarks for these media (such as ER-Ls and ambient water quality criteria) to identify contaminants elevated above levels presumed to

be protective of biological systems. Chemicals which exceed (or nearly exceed) the biological benchmark will be termed "preliminary offshore CoCs".

3. The lists of preliminary ground water CoCs and preliminary offshore CoCs will be compared to identify chemicals in common to both. These will be identified as "proposed offshore CoCs".
4. Preliminary CoCs not common to both lists will be further evaluated for their toxicity, persistence in the marine environment, potential for bioaccumulation, and concentration relative to background levels. Those suspected of posing ecological risk based upon such criteria will be included as proposed offshore CoCs. Other chemicals considered to be of concern by the regulatory agencies and which are not on either list will be included as proposed offshore CoCs as appropriate.

This rationale will be applied on a site-specific basis in each investigation. Proposed offshore CoCs will be subject to regulatory review and concurrence prior to their evaluation in the site-specific ecological risk assessments. Thus, the general work plan should not include a preliminary list of contaminants of concern (COCs), but a list should be included in each Addendum for a specific site.

Other potential stressors pertinent to these assessments include nutrients and pathogens associated with sanitary services for the towns of Middletown, Portsmouth, and Newport, RI. Like classical chemical contaminants, nutrients undergo transport, transformation, and fate processes which affect their ultimate availability to biological systems. The rationale for conducting an analysis of pathogens is as an integrated indicator of nutrient-related stress as well as an indicator of the potential importance of those sanitary services as transporters of contaminants of concern to the area of study. The use of pathogen data in marine ERA's is not wide spread, although pathogen data was used in the Phase I Risk Assessment Pilot Study (Munns et al., 1991) and for the 106 mile Ocean Disposal Site (EPA, 1986a). In the latter study, EPA noted that municipal waste water sludges, for example, contain a wide variety of bacteria, viruses, protozoa, helminths and fungi which do pose potential risks to both the environment and human health, such that risk assessments should include consideration of this stressor.

This reference will be included in the appropriate places. Water column concentrations of nutrients are of primary concern in aquatic systems. A typical direct response to alterations in the availability of nutrients is a shift in plant species' abundances. Indirect effects may ramify throughout consumer trophic levels, resulting in changes to overall community structure and ecosystem function. Sources of these stressors are also expected to be sources for the more conventional chemical contaminants.

In addition to chemical stressors, concern has been raised that low dissolved oxygen (DO) concentrations may impart additional stress on benthic and epibenthic communities in coastal embayments of Narragansett Bay (C. Deacutis, Narragansett Bay Project, *per. comm.*). Low DO can be a stressor itself, as well as an indicator of other stress (e.g., high biological or chemical oxygen demand) within the system. High concentrations of unionized ammonia associated with anoxia and hypoxia may be toxic to aquatic organisms. Possible ecological stress associated with reduced DO will be evaluated in this assessment. Other stressors include physical burial of benthic communities by released blasting grit, miscellaneous debris dropped from docks and ships, and other disturbances associated with Navy operations.

As appropriate to the site-specific investigation, a variety of water column parameters can be evaluated using multi-sensor water quality monitoring equipment. This equipment can simultaneously measure depth profiles of temperature, salinity, pH, conductivity, turbidity, dissolved oxygen, and ammonium. This equipment will allow rapid assessment of the importance of low DO and ammonium as environmental stressors.

2.1.2.2 Selection of Ecological Systems/Species/Receptors of Concern

The rationale for identifying ecological systems/species/receptors of concern (hereafter termed "receptor of concern") at each site under investigation involves evaluation of the importance of the receptor to the ecology of Narragansett Bay, its sensitivity to stressors associated with the site, and its aesthetic and/or commercial importance as a natural resource of the bay. The site characterization for Narragansett Bay provided above identifies a number of estuarine systems and habitat types located in the lower bay. The nature of chemical stressors originating from NETC and NCBC sites suggest several of these to be potentially at risk, including:

- o nearshore habitats directly adjacent to past disposal areas;
- o pelagic communities, including plankton and fish;
- o infaunal benthic communities in sediment depositional areas;
- o soft and hard bottom epibenthic communities; and
- o commercially, recreational, and/or aesthetically important natural resource species.

Added to this list are ecological systems involving critical habitats, such as eelgrass beds, seal haul-out rocks, bird rookeries, and unique spawning areas. Although French *et al.* (1992a, b, c) provides a bay-wide perspective of habitat types, the lack of information concerning critical habitats in immediate association with past disposal

areas in the Lower East Passage and other potential sources of stress represents a data gap which will be addressed in this study.

This list of estuarine systems and habitats potentially at risk from Navy disposal sites leads to identification of target receptors of concern in site-specific ecological risk assessments. Table 2-4 identifies target receptors important to this program. Each of these meets one or more of the criteria identified above. Many of these are important resources species in of themselves for Narragansett Bay, but also can be considered surrogate receptors for larger groups of species. For instance, the hard shell clam is not only an important commercial species for Rhode Island, but also can be considered an indicator species for infaunal bivalves in general. Site-specific investigations will draw upon this list of target receptors of concern as appropriate. Not all of these will be present (and therefore important) at each site. For instance, nearshore, highly weathered habitats associated with some sites may not be suitable for soft shell clams due to the unavailability of appropriate substrate (fine grained intertidal sediments). Rationale for inclusion or exclusion of individual receptors of concern at each site will be included in the site-specific addendum for that site.

Stressors initially introduced to the Bay may potentially affect avian and/or terrestrial systems, including human populations. For example, shellfish contaminated with chemicals or pathogens may be consumed by shorebirds and other animals, resulting in direct or indirect biological effects. In this case, the exposure pathway model for avian aquatic predators assumes multiple prey species as is appropriate for red-breasted merganser and great blue heron. The extent to which the exclusively piscivorous habit of some avain predators, such as osprey, reduces or enhances risk to this target species will be addressed in the risk characterization for each site-specific study.

The proposed offshore ecorisk studies are also supportive of investigations of the potential impacts to humans and terrestrial wildlife from exposure to contaminated seafood (plants, finfish and shellfish) through the contribution of data concerning contaminant concentration in media hypothesized to be part of the exposure pathways to these receptors. While it is not envisioned that measurement endpoints directly evaluating the effects of CoCs on these species will be included in site-specific studies, ecological risks to non-aquatic receptors (such as avian predators) may be evaluated as appropriate. Such target receptors of concern are also included in Table 2-4.

2.1.2.3 Selection of Assessment and Measurement Endpoints

Based upon the preliminary considerations of stressors, their potential ecological effects, and ecosystems which may be at risk, and in keeping with the requirements of the RI/FS process, a suite of assessment endpoints were identified as being of primary concern in this program. As indicated in Table 2-5, these include the

status of each of the ecological systems identified above, as well as the general quality of estuarine sediments and water.

Direct measurement of all assessment endpoints is not possible. Several measurement endpoints will therefore be employed on a site-specific basis as indicators of these higher level ecological values (Table 2-5). These have been selected based upon their relevance to the assessment endpoint and receptors of concern, their relevance to expected modes of action and effects of CoCs, their relevance to determination of adverse ecological effects, the availability of practical methods for their evaluation, and their utility in extrapolations to other endpoints.

Taken together, the measurement endpoints listed in Table 2-5 define the data collection activities to be conducted during the Analysis component. Most of these measurement endpoints have been used in other studies, and have proven to be informative indicators of ecological status in marine and estuarine systems with respect to the stressors identified as important in this assessment. Many serve a dual purpose in that they provide information relevant to two or more assessment endpoints. For example, bioaccumulation of contaminants by blue mussels addresses both general water quality, as well as stressor effects on epibenthic organisms. Several provide insight to the condition of valued natural resource populations, such as is the case with endpoints addressing lobster and hard shell clam abundance, condition, and contamination. Benthic community data serve as indicators of not only chemical stress but physical disturbance as well, particularly in the nearshore habitat zones of landfill environments. These measurement endpoints will be used as an additional weight-of-evidence in the effects assessment component of the risk characterization summary.

In addition to the target measurement endpoints used to evaluate the occurrence of or potential for adverse ecological effects, target exposure point measurements will be employed to evaluate exposure conditions. Shown in Table 2-6, are a list of the exposure indicators which may be measured as part of the exposure assessment component of the ERA. These parameters include bulk chemistry measurements made in environmental media (water, sediment, pore water, biota), as well as geochemical attributes of exposure media which may influence the availability of contaminants to receptors. Indicators also include pathogens which are microbial organisms whose abundance is measured as the concentration of pathogen per unit of matrix; e.g. no./ml, no./g wet tissue. The presence of selected "pathogen" indicators, are actually surrogates for the true pathogens (e.g. enteric viruses), as it is assumed that the presence of the indicator implies the likely presence of the pathogen (Cabelli, 1978).

Chemical markers are defined as source-specific indicators of stressor exposure, i.e., compounds that provide information on the relative importance of various pollutant sources to the environment. For example, coprostanol, a fecal sterol chemical marker,

has been used as a indicator for the relative contribution for sewage inputs into various waters of Narragansett Bay, assuming the concentration of other unmeasured compounds would be available in proportional amounts to the measured indicator. Similarly, selected benzotriazoles have been employed as markers for chemical inputs by specific industries into sediments of this estuary.

2.1.3 Development of Conceptual Models

The above considerations lead to a three-tiered generic conceptual model of potential ecological risks associated with the Navy disposal sites in lower Narragansett Bay. The initial three tiers describe stressor origin, transport, fate, and effects at different spatial and temporal scales: 1) the general down-stream gradient of chemical contamination, 2) initial release and transport of site-specific CoCs to the bay from Navy sources, and 3) longer-term transport, fate, and effects of those CoCs. Conceptual models which identify specific receptors and stressors relative to each site as the fourth and final tier will be included in the site specific addenda. The model is formulated as a set of working hypotheses, and is subject to modification as new information becomes available as a result of data collection activities. The generalized conceptual model will undergo rigorous review by risk managers, scientific peers, and the public to ensure that all concerns have been addressed, and that the assessment will yield a scientifically sound and credible analysis of risk.

The first tier of the conceptual model describes the general down-Bay gradient in stressor concentration described earlier (Figure 1-2). Although many sources contribute to this gradient, and local sources may influence specific stressor concentrations anywhere in Narragansett Bay, this model suggests that contaminant concentrations in the immediate vicinity of Navy disposal areas should be evaluated within the context of the lower Bay to evaluate the extent and significance of Navy disposal areas on the ecology of the Bay. Reference stations located at the geographic extremes of the Lower East and West Passages of the bay provide baseline indicators of risk under relatively pristine conditions.

The second tier of the conceptual model describes the local release of contaminants from Navy sites to the lower Bay (Figure 2-8). Contaminants are transported from land-based sources to adjacent coves and Narragansett Bay predominately *via* surface and ground (seep) water routes, although transport of chemical pollutants bound to soil and dust particles also may occur. Direct dumping and spills may also be important. The geographical configuration of nearshore embayments (Coddington Cove, Coasters Harbor, McAllister Point Cove, Allen Harbor), and resulting hydrodynamic patterns, lead to two hydrodynamically-connected spatial subsystems: nearshore coves and the greater Narragansett Bay. The coves are generally outside the main flow of tidal currents in the Bay, and typically represent areas of sediment deposition immediately adjacent to the disposal areas. Contaminants released into the coves likely experience a longer residence time than

do those released elsewhere in lower Narragansett Bay because of the varying degrees of restricted flushing. Transport out of nearshore coves to lower Narragansett Bay is limited by the rate of cove flushing. Based upon the limited hydrographic information available at this time, this transport is expected to be low level, except perhaps during periods of extreme weather. Thus, a localized gradient would be expected in contaminant concentrations, with highest levels occurring in the inner cove areas nearest to the source. Such a gradient was observed by Quinn *et al.* (1994) in Coddington Cove. Biological transport probably is of lesser importance to Bay-ward movement of contaminants.

The third tier of the model provides details of the aquatic behavior of contaminants leading to exposure of ecological systems, and aides in identification of potential adverse ecological effects. Upon their introduction to nearshore coves, the short-term behavior of contaminants in the water column depends upon their solubility, degradation rates, and sorption to particulate matter (Figure 2-9). Among the divalent metals, nickel, copper, cadmium and chromium (6+) have relatively high solubility and hence will have higher dissolved phase concentrations at similar loadings than many of the organic contaminants and relatively insoluble metals (silver, lead, zinc, chromium (3+)). Individual molecules will sorb and desorb in a dynamic fashion, maintaining an apparent equilibrium relative to sorption state. Dissolved contaminants are transported to other parts of the estuary by prevailing patterns of current. Bound contaminants will be transported horizontally in association with particles, but may also settle to the bottom in localized depositional areas. Once on the bottom, local currents may result in bedload transport of sediment, resulting in a further redistribution of the contaminants. Additional deposition of uncontaminated particles may bury earlier settling particles, removing them from contact with ecological systems. Partitioning dynamics similar to those in the water column will occur in the sediments in response to the geochemical microenvironment of those sediments. Contaminants may be available to biological systems in both the water column and surficial sediments, resulting in biological uptake and/or direct toxicological effects.

In this generalized conceptual model, ecosystems potentially at risk include nearshore habitats, pelagic, benthic, and epibenthic communities, and natural resource species. The description of stressor dynamics suggests risks to these systems to be highest in inner cove areas adjacent to disposal sites. Although risks to other ecological systems present in Narragansett Bay cannot be dismissed, this conceptual model focuses the assessment on ecosystems associated with depositional sediments in these nearshore coves. Stressors important to those systems include metals, organic contaminants such as PAHs, PCBs, butyltins, and OCPs, and perhaps low dissolved oxygen and high ammonia resulting from unknown causes.

At least two important gaps exist with respect to a complete formulation of the conceptual model. Little extant data are available to evaluate the co-occurrence of critical habitats and/or endangered species with stressors potentially originating from

disposal areas. Additional information is also needed with respect to trophic transfer of contaminants. The incomplete tissue residues data set for indigenous biota represents an additional gap. It should be noted that although tissue residues can be used as an indication of exposure, their importance in an ecological risk assessment is currently limited in that evidence linking ecological effects directly with contaminant concentration in tissue (suggestive though it may be) is generally lacking. A more complete understanding of bioaccumulation and trophic transfer is required to evaluate their role in the status of natural resources, and to provide data for evaluating risks to human health associated with seafood consumption. The conceptual model described here is a preliminary representation of our state of knowledge with respect to potential ecological risks associated with disposal areas in lower Narragansett Bay. Site-specific data-gathering activities described in the addenda are designed to provide information with which to refine this model. Such refinement will enhance the interpretation of results obtained during Analysis activities, and will provide a context within which ecological risks will be quantified.

2.2 APPROACH FOR EXPOSURE ASSESSMENT

According to the Framework for Ecological Risk Assessment (U.S. EPA 1992) the analysis phase of an ecological risk assessment consists of characterization of exposure (this Section) and characterization of ecological effects (Section 2.3) (Figure 2-10). Exposure assessment involves quantification of stressor patterns with respect to magnitude, temporal duration and frequency, and spatial scale of occurrence in the environment. Typically, measurement and/or modeling activities are used to define these patterns. Measurement activities may involve attempts to directly quantify the stressor through field sampling programs, or may involve use of indicators of exposure (such as exposure biomarkers). Although generally associated with a greater degree of uncertainty, modeling exercises can be used to predict exposure conditions which cannot readily be measured. Models also permit enhanced understanding of the processes involved in determining stressor patterns, and prediction of patterns under different exposure scenarios.

Attributes of the stressor and of the ecosystem (biotic and abiotic) both influence exposure. Such considerations are particularly cogent when defining the degree of co-occurrence between the stressor and the particular ecological system of interest (e.g., individual organisms, communities, etc.), and therefore the potential for exposure. For example, a metal contaminant may be measured or predicted to occur in depositional sediments, but sediment characteristics (e.g., high acid volatile sulfides) may inhibit metal bioavailability to benthic species. Also, species-specific behavior can produce species-specific differences in exposure within the same exposure field. For example, the infaunal tube dwelling amphipod, *Ampelisca abdita*, recirculates pore water in its tube thus increasing its exposure to stressors when compared with the hard shell clam, *Mercenaria mercenaria*, which does not directly recirculate pore water.

The transport, transformation, and fate characteristics of chemical contaminants in marine and estuarine systems have been the focus of considerable investigation over the past several decades. Although aspects of contaminant behavior are complex and not completely understood, a simplified description is that they either remain in a dissolved state following introduction to a body of water, or they will become associated with water-borne particulate material which ultimately settles in depositional areas. Individual chemical species differ with respect to their degree of affinity to the particular-bound phase. For instance, hydrophobic organic contaminants generally associate quite rapidly with organic matrices on the surface of particles, whereas hydrophilic contaminants remain in a dissolved state nearly indefinitely. In either state, chemical stressors may be transported by prevailing water currents to locations removed from the original source, and may be transformed from their original state through geochemical and biological processes.

For biological effects to be associated with chemical exposure, the biological system must co-occur with the chemical stressor(s). Even with high exposure, the contaminants must be bioavailable to have a direct effect. Bioavailability is influenced by a number of factors, including the degree of sorption to particulates and other surfaces. Organisms can be exposed to these stressors through various routes, including dermal and respiratory contact, and ingestion of contaminated food. Once exposed, biological systems can experience a range of direct toxicological effects, the ramifications of which may be manifested at all levels of ecological hierarchy. Indirect effects, such as trophic transfer, also can result from exposure to chemicals which bioaccumulate.

In the initial evaluation of stressors potentially impacting the Bay, it is recognized that several potential sources exist in lower Narragansett Bay. Among the more important of these sources with respect to this program include:

- o Navy disposal sites
- o Sewage treatment facility outfalls at Coasters Harbor and Conanicut Island (the Jamestown sewage treatment facility), potential sources of nutrient, pathogen, and chemical stress. Pathogens can potentially cause adverse biological effects. For example, a protovirus is suspected to induce soft shell clam neoplasia. Pathogen occurrence and abundance can covary with other source specific stressors and therefore serve as an indicator of stress
- o Industrial and commercial operations in the watershed (particularly the Cities of Newport, Middletown, and Portsmouth, RI) which introduce chemical and thermal stress

Additionally, nonpoint sources to Narragansett Bay (including runoff and combined sewer outfalls) and activities such as fishing and boating all potentially contribute to the introduction of chemical, physical, biological, nutrient, and pathogen stress to the system.

Within each site-specific evaluation, the Navy disposal site being investigated will be considered the primary source of chemical exposure to Narragansett Bay. Exposure assessment in these studies will involve an evaluation of the site-specific conceptual model with respect to hypothesized exposure pathways, and will include direct measurement of key exposure point concentrations along those pathways. Target chemical contaminants identified in Section 4.0 will be quantified in these analyses. In addition to direct measurement of chemistry, other measurement endpoints (tentatively identified in Table 2-5) will be assessed to aid in the interpretation of chemical exposure conditions.

Other site-specific exposure analyses may be conducted as appropriate. These include application of sub-bottom profiling and other geotechnical survey technologies, deployment of hydrographic and current measuring instrumentation, and development and application of contaminant transport models. The value of these technologies in providing data useful for assessing exposure at any given site will be site-dependent.

Also cogent to site-specific exposure assessments will be the availability and quality of exposure information derived from previous investigations. Accompanying the use of these data will be a discussion of the comparability of the various data sets. Finally, presentation of results of exposure assessments conducted at each site will be accompanied by discussions of the uncertainty associated with the exposure analyses.

2.3 APPROACH FOR ECOLOGICAL EFFECTS ASSESSMENT

Ecological effects are quantified by determining the relationships between relevant exposure patterns and resulting responses of ecological systems, in terms of the measurement endpoints identified during Problem Formulation. As with analyses of exposure, both measurement and modeling activities are useful in this process. Several approaches may be used to establish effects profiles, ranging from identification of toxicity thresholds (e.g., sediment and water quality criteria, LC₅₀s, etc.), to development of stressor-response models. This latter approach relates the degree of response observed in the measurement endpoint to the level of exposure experienced by the target receptor. They provide a means of quantifying effects over a range of exposures, and incorporate natural variability in response thresholds. Stressor-response models can be developed from available data, or generated in the course of laboratory and/or field investigations.

Ecological effects assessment activities in site-specific investigations will be determined primarily through the selection of assessment and measurement endpoints

in Problem Formulation. They will consist of literature-reported evaluations of the known effects of site CoCs on the receptors of concern (or suitable surrogates), direct measurement of the toxicity of exposure media to appropriately sensitive marine species through the use of standardized toxicity tests (and development of exposure-response relationships as appropriate), site-specific investigations of the abundance and condition of receptors of concern, and collation of existing toxicity-based criteria and standards for exposure media identified in hypothesized exposure pathways (the fourth tier of the site-specific conceptual models). Specific ecological effects assessment activities to be conducted at each Navy site are identified in the addenda to this Work Plan. Finally, presentation of results of exposure assessments conducted at each site will be accompanied by discussions of the uncertainty associated with the ecological effects analyses.

2.4 APPROACH FOR RISK CHARACTERIZATION

The final step of each ecological risk assessment involves synthesis of the exposure and ecological effects information to determine the likelihood of occurrence of adverse ecological effects (Figure 2-11). Depending upon the nature of information obtained and types of analyses conducted, estimates of risk may be either qualitative or quantitative. Examples of qualitative, screening-level assessments include those which compare single estimates of exposure to an ecological benchmark concentration (e.g., a water quality criterion). If the ratio of the two exceeds some predetermined level (typically 1.0), ecological risk is presumed. Although widely used when more detailed exposure and effects information is lacking, such quotient methods (Barnhouse *et al.* 1986) offer little in the way of evaluating the probability that an adverse effect has or will occur. Weight-of-evidence approaches are also important in ecological risk assessments. Often, definitive linkages between exposure conditions to specific ecological effects cannot be established in a direct cause-and-effect relationship. In such cases, the scientific evidence may suggest possible causal relationships.

More desirable approaches to quantifying risk include those which compare distributions of exposure and ecological responses. When risk is defined simply as the joint probability of exposure and effects, these methods incorporate variability in both stressor concentration and ecological response. In expressing risk as a probability (between 0 and 1), they also obviate the problems associated with open ended risk quotients. Another accepted approach to estimating risk involves simulation modeling. This approach incorporates knowledge of ecological processes directly into risk quantification, and can utilize information regarding both variability and uncertainty in parameter estimates. Probabilistic estimates also result from this method of risk characterization.

Regardless of the approach taken to estimate risk, it is important to establish uncertainties prior to communicating assessment results to the risk manager. This

analysis provides insight to the degree of confidence which should be associated with the estimate of risk. It also serves to evaluate the effects of uncertainty on the entire assessment, and ideally identifies approaches which can be taken to reduce uncertainty. Uncertainty analysis often leads to additional research to enhance understanding of environmental processes and systems.

A weight-of-evidence approach will be used as the primary method for characterizing site-specific ecological risks associated with Navy disposal areas in lower Narragansett Bay. Several lines of evidence will be evaluated in drawing conclusions concerning risk:

1. *Observed adverse effects - comparison with reference stations.* The sampling and analysis designs for each site will provide data from areas adjacent to Navy disposal sites (Derecktor Shipyard, McAllister Point Landfill, Old Fire Fighting Training Area, Allen Harbor) and from appropriate reference stations in the lower Bay. Reference stations with physical characteristics as similar as possible to the sites under investigation will be selected. Statistical comparisons can be made of exposure and ecological effects data collected at Navy *versus* reference areas, treating sampling stations within an area as replicates. Additionally, comparisons may be possible among individual sampling areas as a result of the replication of stations within sampling areas. The latter comparisons will take into account the ecological characteristics of each sampling area, that is, data obtained from sampling depositional areas will be compared with information from like sampling areas. These comparisons will aid in the assessment of risks associated with Navy disposal sites within the context of the general down-Bay gradient in contamination discussed earlier. Temporal interpretation of data is also important. In particular, the seasonal variation of AVS is a critical parameter to determine in assessments involving sediments as an exposure point. Due to the marked decrease of metabolic activity in sulfate reducing bacteria at low temperatures (<10°C), AVS concentrations are at a seasonal minimum during the winter. Because of this, the Navy plans to measure AVS values during the winter months and use this as a conservative indicator.
2. *Analysis of CoC concentration versus observed adverse effects.* Analyses will be conducted to evaluate the relationships observed between measured CoC concentration and the quantified response of the measurement endpoint. For instance, if a particular CoC is causative in ecological impacts to a particular receptor, then a change in the response of measurement endpoints associated with that receptor should be observed with increasing CoC exposure. Interpretation of these patterns will involve a discussion of whether the observed ecological effect is expected to result from elevated exposure to the CoC.

3. *Analysis of bioaccumulation.* Elevated tissue residues in target receptor species will be interpreted as an indication that CoCs are bioavailable and can potentially be transferred in other receptors through trophic interaction. Analyses may be included on a site-specific basis to assess trophic transfer of CoCs to receptors not directly sampled. When possible, information from the literature will be used to estimate ecological risks to receptor species resulting from the presence of CoCs in tissues.
4. *Analysis of toxicity evaluations versus observed ecological effects.* Results of toxicity tests conducted on exposure media collected at field sites will be compared with measurement endpoint response at those sites. Through identification of the exposure pathways and appropriate measurement endpoints, care will be taken to ensure that toxicity endpoint-measurement endpoint comparisons are appropriate for a particular receptor.
5. *Comparison of exposure point concentration with toxicity-based criteria and standards.* As described above, hazard (risk) quotients (HQs) are simply the ratio of measured or expected exposure levels with appropriate ecological benchmarks. Benchmark concentrations may be federal or state water quality criteria, sediment toxicity or management standards, or other standard of environmental quality. Johnston (1994) applied the hazard quotient approach to identify adverse contaminant levels as part of the estuarine ecological risk assessment conducted for Portsmouth Naval Shipyard. This screening analysis used specific evaluation criteria based on water quality criteria, literature toxicity thresholds, Washington State sediment management standards, crustal weathering models, SEM/AVS ratios for divalent metals, and pore water equilibrium partitioning for organic contaminants to relate to direct measures of toxicity. Developed for use in identifying media protection standards for sediments and surface water, this approach is a useful construct for evaluating ecological risks using quotient methods. In addition to calculation of HQs for each CoC in exposure point/receptor pairing, HQs will be summed across CoCs to calculate hazard indices (HIs). HIs will be used as measures of the summed effects of all CoCs present in environmental media, assuming additivity in CoC interaction.
6. *Comparison of exposure point concentration with toxicity data.* Based on the known adverse effects of CoCs as reported in the literature and in toxicity data bases (e.g., AQUIRE), the concentrations of CoCs measured at critical exposure points will be evaluated against suspected effects levels. Elevations above such levels would implicate the CoC in causing risk.

In addition to comparisons made within the data set generated as part of this

study, relative risks can be evaluated through comparisons made with the findings of other risk assessments conducted in the marine environment. Previous work in the Davisville-Quonset study area (Munns *et al.* 1991, 1992,1994a) provides valuable information concerning environmental conditions in other areas of lower Narragansett Bay. Incorporation of information from this and other environmental assessments permits evaluation of the ecological risks associated with Navy facilities in lower Narragansett Bay to be conducted on a holistic basis.

This weight-of-evidence approach will be used to evaluate causal relationships between CoCs (exposure) and the existence or suggestion of adverse ecological effects. For example, the observation of anomalies in benthic community structure in areas with SEM/AVS ratios greater than 1.0, but low organic CoC levels, would suggest divalent metals to be posing ecological risk in those areas. Observation of toxicity of bulk sediments collected in those areas would further support this hypothesis. Conversely, benthic community structure anomalies in the absence of elevated CoCs and sediment toxicity may implicate other types of stress, such as physical disturbance or low near-bottom dissolved oxygen. All available evidence will be utilized in evaluating the lines of evidence relating CoC exposure to potential adverse ecological effects. It should be noted that not all lines of evidence need point to one or more CoCs as causative agents for risk to be presumed in association with that CoC. In this weight-of-evidence approach, it will only be necessary to have the preponderance of evidence suggest a causal relation in CoC-receptor pairings for risk to be concluded.

The weight-of-evidence approach will be augmented when possible through the use of simulation modeling and joint probability analyses. As an example, joint probability analyses have been used to quantify risks associated with the Allen Harbor Landfill (Munns *et al.* 1994a), and will be included in the characterization of risks for that site. Similar analyses may be feasible in other site-specific investigations with the collection of appropriate data. Such approaches will be identified as appropriate in the site-specific addenda prior to their implementation.

The uncertainties associated with risk characterization activities, and therefore with the entire site-specific ecological risk assessment, will be discussed and quantified (if possible) when investigation results are reported. These discussions will include identification of assumptions used, any remaining data gaps, and the limitations of the assessment.

3.0 DATA QUALITY REQUIREMENTS, COLLECTION, AND ANALYSIS

Of obvious importance to all measurement and monitoring projects (*i.e.*, data generating activities) is the establishment of objectives for data quality based upon the proposed uses of the data (Stanley and Verner 1983). A primary purpose of a quality assurance program is to maximize the probability that the resulting data will meet or

exceed the DQOs specified for the project. Normally, DQOs are established in terms of five aspects or attributes of data quality: precision, accuracy, completeness, representativeness, and comparability (Stanley and Verner 1983). The following subsections provide definitions for each of these attributes in relation to the bioassay and chemistry data to be collected for site-specific work plans.

3.1 PRECISION

Precision is defined as the degree to which individual measurements converge upon a single value. It can represent an estimate of the random error inherent in the sample population being measured (variability), or in the measurement process itself (uncertainty). Precision in the environmental sciences usually is quantified through measurements of multiple sampling units (e.g., field sediment samples, replicates of a laboratory treatment, etc.). Instrumental precision often is determined through multiple measurements of a single sampling unit.

Bioassay data. The precision of bioassay results will be evaluated in two, conceptually similar, ways: by repeating individual tests (test precision) and through replication of treatments within a single test (treatment precision). The first approach addresses error associated with test conditions and variation in individual organism response, whereas the second primarily addresses variation in response. Test precision of the toxicity bioassays has been measured using a standard toxicant in a systematic fashion (SAIC unpublished data; Morrison *et al.* 1989). Such results provide a basis for setting criteria for acceptable test performance. Levels of test precision, expressed as the coefficient of variation (CV) of endpoints associated with exposures to cadmium chloride (Cd) for *Ampelisca*, and copper sulfate (Cu) and sodium dodecyl sulfate (SDS) for *Arbacia*, are presented in Table 3-1. Based upon this information, precision data control charts will be generated and successive standard toxicant toxicity test results plotted on the chart to ensure that the data fall within the limits of quantified variability. *Ampelisca* test acceptability also is determined based on control response. The acceptable control response level for this bioassay is <10% mortality.

Chemistry data. Precision is determined by the analysis of laboratory duplicates, which are prepared by homogenizing and sub sampling a sample in the laboratory and carrying the sub-samples through the entire analytical process. Precision will be expressed as the relative percent difference (RPD) for all laboratory duplicates.

3.2 ACCURACY

Accuracy is defined as the degree of concurrence between measured values and the true or expected value of a parameter. It represents an estimate of the systematic error or net bias inherent in the measurement process. When considered

together, accuracy and precision measure the total error associated with a set of measurements. In the environmental sciences, the true value of a measurement parameter within a sample is seldom known (e.g., the true concentration of a particular xenobiotic chemical in a tissue sample, or the true toxicity of an environmental contaminant to a particular species). Evaluations of accuracy therefore are based upon expectations of the true values. Estimation procedures can be used to establish such expectations, and important information can be obtained through comparisons of different measurement approaches. Accuracy of analytical measurements can be determined through evaluation of results obtained from quantitation of "known" material (e.g., standard or certified reference material, spiked material, blanks).

Bioassay data. The accuracy of a toxicity test is not measurable because the true toxicity of any given environmental sample is unknown. Further, toxicity is a relative property dependent upon exposure conditions (test duration, bioavailability of contaminants) and the species being tested.

Chemistry data. Accuracy is verified by the analysis of reference materials, intercalibration samples, internal standards, procedural blanks, initial calibration, calibration checks and matrix spikes.

3.3 COMPLETENESS

Completeness is defined as "a measure of the amount of data collected from a measurement process compared to the amount that was expected to be obtained under the conditions of measure" (Stanley and Verner 1983). An aspect of completeness that can be expressed for all data types is the percentage of valid data (i.e., not associated with some criterion of potential unacceptability) obtained from the measurement process. It may also be expressed as the percentage of samples for which valid data are obtained. Reasonable target completeness values are 90% for samples analysis. The inability to complete a sample analysis is documented in the laboratory notebook with an appropriate explanation.

To ensure that all required samples are analyzed, each sample is assigned a unique identity that is tracked through all stages of an experiment: from assignment of ID to calculation of final concentration(s). The analyst conducting the experiment is responsible for ensuring that 100% of the samples have been completed, or (in the event of sample loss) maintaining records that document the loss. The work assignment manager is responsible for verifying the records of completeness.

3.4 REPRESENTATIVENESS

Representativeness is defined as "the degree to which the data accurately and precisely represent a characteristic of a population parameter, variation of a property, a process characteristic, or an operational condition" (Stanley and Verner 1985). In

the context of this project, representativeness refers to the degree to which the chemical and biological data reflect actual environmental conditions in the central and southern areas of Narragansett Bay.

Bioassay data. Over the course of methods development and subsequent use in numerous environmental projects conducted for the U.S. EPA and others, the *Ampelisca* and *Arbacia* bioassays have been shown to produce toxicity information which is representative of benthic and water column organism responses to environmental contaminants. Species used in both tests are sensitive to the suite of contaminants suspected to be associated with the Derecoctor Shipyard. Although these bioassays are considered to be "acute" or "rapid", the results of these bioassays may be indicative of the longer-term, chronic effects of introduced compounds. *Mytilus* contaminant bioaccumulation and growth have also been shown to be sensitive indicators of contamination and stress. Although few biomonitoring approaches to assessing chronic water quality problems exist, data obtained from this test are considered to be representative of long-term responses of pelagic marine species.

Chemistry data. Representativeness will be addressed by the proper handling and storage of samples prior to analysis and analysis in a timely manner so that the material analyzed reflects the original material collected as accurately as possible.

3.5 COMPARABILITY

Stanley and Verner (1985) define comparability as "the confidence with which one data set can be compared to another". For this work plan, comparability of data will be maximized because the technical approaches to be used in this project are based upon scientifically accepted procedures. For example, the procedures to be employed in chemical and biological assessments of sediment and water quality follow protocols identical to those of numerous EPA studies, including the marine ecological risk assessments for NCBC Davisville and the Portsmouth Naval Shipyard. Thus, comparability of assay and test results with these and other studies should therefore be high.

3.6 PLAN FOR DATA COLLECTION AND ANALYSIS

3.6.1 Study Area and Reference Site Selection.

The site specific plans for data collection require the selection of appropriate sampling locations, both within the vicinity of the ecosystem at risk, and at sites remote to the zone of potential impact.

3.6.1.1. Study Area Sampling Locations

The selection of sampling locations will be determined from review of information obtained from prior studies including general monitoring studies, Confirmation studies, Remedial Investigation studies and Project data obtained from earlier phases of the site-specific ERA in process. In addition to these quantitative data, more qualitative information may be obtained from unpublished sources such as knowledgeable commercial and recreational fisherpersons, concerned citizens, general observations from academic and state-associated researchers. The station selection strategy is to 1) fill data gaps; 2) determine nature and extent of contamination, 3) establish exposure-response relationships, and 4) confirm suspect results from earlier studies. As each site-specific addenda is developed, stations will be proposed along with station-specific rationale for selection to the Ecorisk Advisory Board for consideration.

3.6.1.2. Reference Site Sampling Locations

Reference stations are required to develop comparative information needed for the risk assessment. Specific reference locations have been selected to cover the range of hydrographic, depositional, physical flushing, and habitat quality characteristics of the areas directly adjacent to the disposal areas. The reference stations also provide a means of evaluating the source strengths and ecological effects associated with other, non-Navy stressors operating within the study area. Non-Navy stressors include sources of pollution from sewage treatment plants, storm water runoff, marinas and recreational boating, as well as Bay-wide ecological stress as a result of red tides, prolonged temperature maximums, and other climate-related phenomena. In addition, data from reference stations allow the relative contribution of stress from Navy disposal sites on aquatic resources within the Lower East Passage study area to be inferred. Reference sampling areas are located at the north end of Conanicut Island at North Jamestown (NJ), a station used previously in a number of studies (including the marine ecological risk assessment for Allen Harbor (Munns *et al.* 1991, 1992, 1994a)); within Potter's Cove in Jamestown (JT), directly across from NETC; in Newport Harbor (HN); and just outside the East Passage in Rhode Island Sound (RIS). For example, the rationale for the McAllister PT/NCBC Davisville reference site at Jamestown's Cranston Cove is as follows:

- o It has a localized, small suburban fresh water influx similar to the environs of NETC Newport,
- o It has modest eel grass beds in shallow water as does NETC Newport, and
- o It is at approximately the same latitude and is similarly exposed to the general down bay gradient of contamination that the environs of

McAllister Point would experience in the absence of the effects of the landfill.

The remainder of this sampling plan describes the general approach for data collection activities which may be conducted during site-specific ERA's.

3.6.2. Biota

3.6.2.1 Target Species Collection

A variety of indigenous biota samples may be collected to evaluate specific measurement endpoints for bioaccumulation and direct exposure to the chemicals of concern. These target species have been selected to represent a range of ecological functions (trophic, phyletic, interaction, etc.) within the ecosystem, as well as being components of commercially important aquatic resources in Narragansett Bay. The biota data can also be used to evaluate trophic transfer of contaminants and to assess the potential impact to human health from seafood consumption. The species which have been proposed encompass a range of habitat type, feeding mode, mobility, and trophic position (including commercially important aquatic resources) within the nearshore ecosystem and include:

1. Hard shell clams (*Mercenaria mercenaria*, *Pitar morrhauna*): Abundant and ecologically important filter feeders of sub-tidal environments and common food sources for higher marine trophic species as well as humans.
2. Mussels (*Mytilus edulis* or *Modiolus demissus*): Abundant and ecologically important filter feeders of sub-tidal and intertidal environments including marshes; common food sources for higher trophic species including lobster, crab, and birds; occasional food sources for human consumption.
3. Soft shell clams (*Mya arenaria*): Abundant and ecologically important filter feeders in localized mudflat environments, common food sources for higher trophic species including crabs and birds; common food sources for human consumption.
4. Lobsters (*Homarus americanus*): Abundant and important predator/scavenger species of sub-tidal environments, common food source for higher trophic marine species including striped bass and harbor seals, and common food sources for human consumption, typical range 10-100 m.
5. Mummichogs (*Fundulus spp.*): Abundant and ecologically important scavenger/detritivore fish species in nearshore marsh environments, and common food sources for higher trophic species including fish, crabs and birds; typical range 10-100 m. Exposure data on this fish species will be used

to assess exposure to other benthically-coupled fish species such as winter flounder.

Of the above group, species 1, 2, 3, and 5 are expected to be abundant at NCBC Davisville, while species 1, 2, and 4 should be readily available at the NETC. The State has suggested possible replacement of mummichogs with nereid worms. We would recommend against this option, as the mummichog is an important food chain species, despite having a wide range of motion.

The State of Rhode Island has requested collections of oysters, because of significant sets found in Allen Harbor. Other bivalves are favored because of current harvesting practices and the perceived likelihood that these species would support a more sustainable fishery than would the oysters. Each site will be evaluated separately to determine endpoint species to be used, based on species availability at that site.

Estimates of abundance, and distribution within the study area can be developed for each of the species based on field observations and historical data. Chemical analyses of whole soft tissue can be conducted on: hard shell clams (*Mercenaria*, *Pitar*), blue mussels (*Mytilus edulis*), soft shell clams (*Mya arenaria*), and mummichogs (*Fundulus* spp.). Separate analyses of lobster (*Homarus americanus*) muscle (claw and tail), hepatopancreas and reproductive material ("tamali") may be necessary because known variation exists in chemical accumulation between these tissues, and to support analysis of different trophic transfer scenarios in human health calculations. Shell and exoskeletal material will not be analyzed for any species. Condition indices can be evaluated for all bivalve species, soft shell clams will be evaluated for the presence of hematopoietic neoplasia (Hn), and mummichogs will be inspected for external evidence of pathological damage (fin rot, gill lesions, etc.) Benthic diversity analyses can be conducted to assess the health and condition of the benthic invertebrate community.

3.6.2.2 Mussel Deployments

Deployed mussels (*Mytilus edulis*) have been used effectively to characterize water column exposure conditions and evaluate potential ecological effects. The following paragraphs describe the typical approach for conducting deployments. An apparatus consisting of moorings, anchor weights, and four mussel cages will be deployed at six to eight stations within the study area (Appendix Table A4). Data are collected for chemical residue levels and individual growth.

3.6.3. Sediment Collections

Sediment samples will be collected for toxicity and chemistry analysis. The use of bulk sediment pore water, and depth intervals will be determined on a site-specific

basis. The exact approach for each site is presented in each appropriate addenda to this work plan. In the sections below, a general description of the collection methods are described.

3.6.3.1 Deep Cores

The purpose of the deep cores is to develop information of the depositional environment within the sampling areas, estimate rates of sediment and contaminant accumulation, identify historical contamination patterns, determine the extent of contamination with depth, and evaluate geochemical transformation processes. Piston cores are used to take deep (≈ 1 m) cores.

A standard piston corer, the biological corer, is used to retrieve cores from intermediate water depths (<10 m). The corer uses polycarbonate tubes and is deployed using a series of 3 meter long extension rods to push the corer into the sediment. Cores up to one meter long are recovered using this design. Biological cores are stored in the vertical position and are transported to the lab for further analysis.

A trigger release piston corer, the Nemesis, is used in deeper water (>10 m). The landing pad design has lead weights loaded at the top and triggers upon contact with the sediment/water interface. The polycarbonate core barrel is then forced into the sediment to recover 1 to 1.3 meter cores from any water depth. The Nemesis cores are stored vertically and transported to the lab for analysis, where they are briefly stored at 0°C, sectioned and then stored at 20°C. Both types of cores are sampled by using a vertical extrusion box that extrudes the core out of the top of the core tube in 1 cm (or greater) increments without disturbing the core.

Deep cores about 1-2 m in length may be collected in replicates at selected locations. Each replicate core will be scanned for magnetic susceptibility to determine the representative core (for each sampling area) to be selected for determination of geological and chemical profiles. Core profiles will be obtained by sectioning the core at appropriate horizons to provide samples for analysis of bulk metals, organic contaminants, grain size and total organic carbon. A subset of profile samples will be selected for the analysis of Pb^{210} and Cs^{137} radionuclides necessary for dating the core (Appendix Table A1).

3.6.3.2 Surface Grab Sampling

The purpose of surface grab sampling is to develop information on the benthic habitat characteristics and community structure, chemical exposure levels within the surface sediments, the toxicity and availability of chemicals present, and assess the route and fate of chemical exposure in the surface sediments of the study area. In addition, bioavailability and exposure-response information will be evaluated by

chemical and biological analysis of interstitial water extracted from a subset of samples from the most contaminated/impacted stations.

The investigator should determine the deployment capabilities of the vessel to be used and the equipment available prior to planning the sampling event. The Box-corer, Smith-MacIntyre and Van Veen grab samplers are three devices which may be deployed from a vessel with suitable winches, and an A-frame or davit arrangement. The materials and equipment required or available for sediment sampling may also vary depending on the vessel used for sampling.

Short cores may be obtained from an undisturbed grab sample by inserting "small" (15 cm) cores into the middle of an undisturbed grab sample. In this method, a cap is placed on the top of the core and the core is removed from the grab. A second cap is placed on the bottom of the core after removal from the grab. All caps and liners are precleaned with acid and methanol.

Surficial sediment (top 2 cm or other horizon, as appropriate) of an undisturbed grab sample is sampled using a clean titanium scoop for chemistry and toxicity analysis. Approximately 2-3 Van Veen or 3-5 Smith Mac grabs are sampled to obtain about 3-liter of sediment from this horizon. The surface material is composited in a 12-liter Nalgene bucket, stirred with a titanium stirrer for ~30 seconds, and then subsampled into precleaned containers for organic and inorganic chemistry and toxicity studies. The remaining material in the box cores are sieved for bivalves and any bivalves will be retained for chemical analysis. Additional box core samples may be obtained at each station and used for benthic infaunal analysis.

The scoop is rinsed with distilled water, acid, and methanol between grabs. The grab sampler is "washed-down" with seawater between stations. The samples are stored on ice during collection and at -20°C upon return to the laboratory. A subset of stations will be resampled, based on a combination of factors, primarily the presence of toxicity and/or elevated contamination levels.

3.6.4. Hydrographic Studies

Data recorders are used to develop continuous records of water column conditions. These include current measurements at selected locations, characterization of water column hydrological and chemical oceanographic conditions (dissolved oxygen, salinity, pH, nutrients, chlorophyll, etc.), and developing information to characterize the habitats and aquatic resources within the NCBC and NETC study areas. Depending on seasonal timing, the instrument deployments can last from 4 to 8 weeks.

3.6.5. Biological Testing

3.6.5.1. Toxicity Testing

The acute toxicity of sediments are assessed as a measure of the biological effects of sediment contaminants. The chronic toxicity of porewaters are assessed to evaluate the biological effects of interstitial water (porewater) contaminants to infaunal and epifaunal organisms. Sediment and pore water samples may be collected from the same locations as chemistry and evaluated for toxicity using the 10-day amphipod test and the sea urchin fertilization test, respectively. Organisms collected for toxicological investigations include the benthic amphipod, *Ampelisca abdita* and *Rhepoxinius sp*, for sediment toxicity assessments, and the sea urchin, *Arbacia punctulata*, for pore water toxicity assessments.

Interstitial (pore) water metals will be measured in surface sediment samples utilizing the vacuum extraction method of Winger and Lasier (1994). Duplicate sample preparations are made for pore water toxicity and metals analyses. Approximately 100 ml of pore water can be obtained from sediment held at 4°C in a 24 h period. The concentrations of trace metals will be analyzed from the interstitial water samples and toxicity tests with *Arbacia punctulata* will also be performed on these samples.

A change in the redox state of sediment samples caused by exposure to oxygen is likely to free some sulfide bound divalent metals (Cd, Cu, Pb, Ni, Zn, and Fe) and increase the concentrations of these metal species in pore water samples. A study by Howard and Evans (1993) indicates that the acid volatile sulfide (AVS) concentrations of samples exposed to air upon collection is ~20% of the AVS concentrations of the same samples handled in a nitrogen atmosphere. This decrease is caused by both the loss of H₂S gas and dissolution of solid-phase sulfides upon exposure to oxygen. In this study, the samples will be composited and stirred vigorously in a bucket for one minute to homogenize them prior to subsampling for AVS and pore water toxicity. In the laboratory, the vacuum extraction method of Winger and Lasier, (1991) is used, wherein porewater is removed from sediments with minimal aerial exposure. This method has been found to produce samples of similar toxicity as that of other porewater extraction techniques (Carr and Chapman, 1995). Subsequently, samples are filtered through 0.45 um filtration apparatus for both porewater toxicity and metals chemistry analysis. Although filtration is not necessary for toxicity evaluation, it is performed to maintain comparability with the processing required for metals analysis (because of potential sediment interference in the dissolved phase measurement). Similarly, the loss of AVS during the press sieving of bulk sediments for toxicity testing is also likely. Hence, the laboratory sediment processing procedures causes aerial exposure and a loss of sulfides, although the magnitude of this effect has not been quantified. Therefore, pore water metal concentrations in both bulk sediments and in extracted pore waters are likely to

increase due to dissolution of solid-phase sulfides during sample handling in the field and in the laboratory. The results produced will be more conservative indicators of potential trace metal toxicity problems than results obtained from an alternate method whereby samples are not exposed to oxygen.

Pore water toxicity, simultaneously extracted metal (SEM) and AVS measurements are assumed to be directly comparable given similar sample handling procedures. The comparability of bulk sediment chemistry and toxicity to amphipods is more uncertain, given that sediments for bulk analyses are not press-sieved prior to analysis. These uncertainties may lead to disparate results between toxicity test methods, and such findings will be addressed in the discussion of uncertainty in the Effects Assessment.

Parameters for bioassay methods are indicated in Table 3-3 for the amphipod assay, and Table 3-4 for the Arbacia assay. Sediments from both surface and sub-surface zones may be subjected to bioassay analysis. However, in instances where insufficient sediment can be obtained at depth, extrapolation of toxicological data derived from surface samples may be necessary. This extrapolation would be accomplished using data addressing chemistry, normalizing factors (AVS, TOC, grain size), and surface toxicity, as available.

3.6.5.2. Condition Indices

Condition indices (tissue dry weight-shell length ratios) may be evaluated for bivalve species, and mummichogs may be inspected for external evidence of pathological damage (fin rot, gill lesions, etc.).

3.6.5.3. Benthic Community Structure Analyses

Benthic community structure analyses are designed to evaluate impacts of physical and/or chemical insult on the stability and diversity of indigeneous populations. Given that communities represent higher level organization than the species, this analysis significantly augments results obtained from toxicity analysis.

The goal of the benthic community analyses will be to assess differences in major community parameters between the landfill and reference sites. The parameters examined will include species richness and evenness, the proportion of deposit feeders, density and diversity of amphipods, density of *Oligochaetes*, and density of *Capitella capitata*. Benthic invertebrates will be sampled at locations previously chosen to describe the concentration of contaminants within sediments and organisms as well as the toxicological properties of sediments adjacent to the landfill. These locations will be used for benthic community sampling because of the availability of data on chemistry and toxicology, and to provide information on food chains and rates of sedimentation and bioturbation at the sites. In the intertidal area,

samples will be taken both within and outside of patches of *Mytilus* since they modify the habitat by the structure of their shells and by their biodeposition. Analysis of subtidal organisms planned for May 1995 will be supplemented by data collected at three sites by Menzie-Cura in August 1993.

The sampling plan does not, however, lend itself to sophisticated statistical analysis. The number of stations is small (12 landfill, 3 reference). There is much variability within the landfill area; site reconnaissance indicates that intertidal sites (7 total) are sand and pebble pavements with embedded *Mytilus* and with fresh water seeps at some locations, whereas subtidal sites (5 total) have various amounts of pebble and shells overlying sand and silt sediments. Because of the small sample size and large number of potentially significant natural and landfill associated variables, it is doubtful that cause and effect relationships can be shown statistically. However, the patterns observed will be compared with other exposure and effects measures to provide further weight-of-evidence of linkage (or lack thereof) between exposure and observed effects.

3.6.5.4. Sewage Pathogen Analyses

Sewage is known to contain concentrated numbers of microbial pathogens. Even after rigorous treatment, sewage discharges may still harbor numerous resistant pathogens. Sediment and tissue samples may be collected to investigate the sanitary quality of the marine environment via the types and densities of pathogens present in the sample. The indicators of choice include total and fecal coliforms (including *Escherichia coli*), fecal streptococci and enterococci and *Clostridium perfringens* spores. The selected indicators have undergone extensive study and have been chosen on the basis of meeting several standard indicator criteria. While no one indicator meets all of these criteria, the use of several indicators provide an effective screen for detection and quantification of potential pathogens in shellfish and sediments.

3.6.6. Chemical Analyses

A list of chemical analytes which may be measured in this study is provided in Table 3-2, along with their respective method detection limits. Detailed information on the SOPs and QA/QC program are in Appendix A and B, respectively. Quantification of potential chemical markers in sediments may also be conducted by to identify chemical tags useful in determining unique sources of contaminant input. This work has been a component of ongoing chemical marker research and development at GSO/URI over the past 20 years. The assessment will provide an application to examine the use of organic and inorganic compounds unique to specific sources in description of pollutant transport and fate in Narragansett Bay. This description will be valuable in delineating the relative contributions of each source to any observed environmental impact.

4.0 ANALYTICAL PROCEDURES

This section provides an overview of chemical analytical procedures employed in this study, including methods (Section 4.1.) and quality control procedures (Section 4.2.). Complete descriptions and SOP's are provided in Appendices B and C. Important URI/SAIC SOPs to be used in this project are listed in Table 4-1.

4.1 CHEMISTRY ANALYTICAL PROCEDURES

The bulk of chemistry analyses required for this study will be conducted by GSO/URI, where protocols have been to achieve method detection limits (MDLs) sufficiently low for the DQOs of this project (Table 3-2 and Appendices B and C). They will continue to maintain and improve the quality of their work through the analyses of National Institute of Science and Technology (NIST) performance evaluation samples, standard reference materials, and, as appropriate, sample splits with the NETC onshore study contractor. A complete description of the chemical analytical procedures is included in this document as Appendix B. The Geochemical and Environmental Research Group (GERG) of Texas A&M University will perform analyses of butyltin concentrations in sediments and biota on a subcontract basis (Appendix B). A brief description of the analytical methods to be employed in this project is provided below.

4.1.1. Organic Analyses

Prior to analysis for organic contaminants, samples (sediment or tissue) will be homogenized until uniform and a small aliquot dried to determine the moisture content. Aliquots used for chemical determinations will be spiked with surrogate internal standards and then extracted with acetonitrile under reflux conditions. Water samples (~ 3 liters) from elutriate tests will be spiked with surrogate internal standards and then extracted with methylene chloride. Extracts containing PCBs, PAHs, and OCPs will be passed through silica columns to remove interferences and to separate analytes into different fractions based on chemical structure. PCBs and OCPs will be quantified using a Hewlett-Packard 5890 gas chromatograph (GC) with an electron capture detector (ECD) and a 60-m DB-5 fused silica capillary column. Quantification of PAHs will be accomplished using a Hewlett-Packard 5971 gas chromatograph equipped with a mass selective detector (GC-MSD) and a 30-m DB-5 fused silica capillary column. Selected PCB/OCP samples will be analyzed by GC-MSD to confirm (qualitative) GC-ECD analyses.

Surrogate internal standards are added prior to extraction and they are used for organic quantitation. In addition, recovery internal standards are added just prior to instrumental analysis and they are used to calculate the recovery of the surrogate internal standards. Thus, the organic data is recovery corrected but it also includes

the recovery of the surrogate internal standards so that values can be uncorrected if desired. It will be stated in the summary and text of all reports that data is recovery corrected and that it may be converted to uncorrected by using the recovery of the surrogate internal standards. All data will be included in the reports. Most of the existing organic contaminant data from Narragansett Bay has been generated by one (JGQ) of the principal investigators. All of this data, as well as the Battelle organic data (Battelle, 1994), was obtained using surrogate internal standards for quantitation.

4.1.2. Metals Analyses

As with the organic analyses, sediment and tissue samples for metals analysis will be homogenized until uniform. Sample aliquots will be freeze-dried, weighed, and digested in acid. Matrix spikes will be added to 5% of the samples prior to digestion. Sediments and biota will be digested by microwave. Following digestion, samples will be analyzed by either Graphite Furnace Atomic Absorption Spectrophotometry (GFAAS), or by Inductively Coupled Plasma Spectrometer (ICP).

The Method Limit of Quantitation (MLQ) approach is used in the trace metal studies. In this approach the instrument detection limit (IDL) is the lowest concentration level that can be determined with a 99% confidence from replicate analyses of a blank. The Limit of Quantitative Detection (LQD) is defined as 5 times the IDL for ICP analyses, and 10 times the IDL for GFAA analyses. The MLQ is defined as follows: $LQD \text{ (ug/L)} \times \text{sample volume (L)} / \text{sample dry weight (g)}$. MLQ values for sediments and biota samples are in units of ug/g. MLQ values for water samples are in units of ug/L. The MLQ is the minimum concentration of a substance that can be measured and reported with confidence as a concentration greater than zero.

4.1.3. Chemistry Quality Control Procedures

There are many quality assurance checks and quality control procedures that are routinely performed during all phases of chemistry sample collection and analyses. A general description of some of the analytical QC procedures is provided below.

4.1.3.1 Organic QC Procedures

Laboratory blank analyses. Laboratory blank analyses are conducted with every twenty sediment or tissue samples. Field blanks (water rinsings from the clean collection apparatus for sediments) are also conducted at the rate of one per 20 field samples. These analyses are carried out as usual including the addition of surrogate internal standards to samples followed by extraction and column chromatography. Target organic analytes detected in samples at less than five times the level detected in the blanks are treated as unreliable.

Extraction efficiencies. Extraction efficiencies are measured by conducting spike and recovery analyses using standard mixtures of the analytes added to laboratory blanks or field samples. Matrix spikes are conducted for 1 in every 20 field samples. The spiking levels are similar to those measured in samples from low level and moderately contaminated sites. Background levels of these compounds are also measured in unspiked duplicates for subtraction from the spiked samples. The recoveries are within $\pm 50\%$ for at least 70% of the spike analytes relative to the surrogate internal standards added at the start of the analyses. Recoveries of surrogate internal standards are 30% to 130% based on recovery internal standards added to all samples just before GC injection.

Instrumental detection limits. The instrumental detection limits for the GC-ECD and GC-MSD analyses are between 0.01 and 1.0 ng per component depending on the specific analyte in question. The MDL for individual PCBs and OCPs is 1 ng/g for sediment, and 2 ng/g for tissue (minimum sample dry weight of 5 and 2 g, respectively). The corresponding values for PAHs are 10 ng/g and 20 ng/g, respectively.

Instrument variability. Instrument variability is determined by replicate injections of standard mixtures or samples; the values for all analytes usually range between 1 and 5% RSD (Relative Standard Deviation) for the standards, and between 1 and 10% RSD for the samples. Overall analytical precision is determined by processing duplicates of a randomly chosen field sample (e.g., two analyses of a sample from the same glass storage container). Duplicates are conducted for 1 every 20 field samples (using field samples). The relative percent difference is 35% or less for 70% of the analytes whose concentrations are 10 x MDL.

Analytical accuracy. Analytical accuracy is determined by the analysis of a standard reference material obtained from the National Institute of Standards and Technology (NIST; sediment SRM 1941a and tissue SRM 1974a). One SRM is analyzed for every 20 field samples (using NIST SRMs). In addition, we routinely participate in the NOAA Status and Trends Intercalibration Exercises conducted by the NIST. The repeated analysis of these certified materials allows a check on the accuracy of our analyses, which is within $\pm 30\%$ of the certified concentration range for 70% of the analytes whose concentrations are 10 times the MDL.

In summary, the frequency of the URI QC procedures for organic contaminants is as follows:

- o Blanks - At least 1 every 20 field samples (using laboratory or field blank)
- o Matrix Spike - 1 every 20 field samples (using field samples or laboratory blanks)
- o Duplicates - 1 every 20 field samples (using field samples)
- o SRMs - 1 every 20 field samples (using NIST SRMs).

Thus, a minimum of four QC samples will be analyzed for every 20 field samples processed. If any problems occur or if any of the QC values are outside of defined limits, corrective action will be taken immediately to correct the situation. Correction action may include re-injection, re-extraction, preparation of new standard solutions, changing chemicals and solvents, and repair of instruments.

4.1.3.2 Butyltin QC Procedures

Quality assurance/quality control for butyltin analysis relies on reproducible, precise, accurate and non-contaminating procedures. This is insured by analysis, with each sample set, of a duplicate, certified reference material, reagent blank, and spike blank. Each set of samples run must comply with established limits of acceptability. Due to the fact that the quantitation of each butyltin depends upon its individual chemical properties, different ranges of acceptance are set for each butyltin. The reproducibility, precision and accuracy is not only monitored within a given set of samples, but also compared to other previous analyzed sets.

Tri-n-propyltin (TPT) is used as a surrogate standard added before samples are extracted. The percent recovery of the surrogate is determined by the addition of tetra-n-propyltin before gas chromatographic analysis. Percent recoveries of TPT are monitored and any sample with percent recovery less than 40% is re-extracted.

Potential contamination by butyltins is monitored by running reagent blanks with each sample set. If analytes are detected in the reagent blank at concentrations greater than three times the MDL, the entire set is re-extracted. Butyltins used to make standards, surrogate and external standards are checked for purity. Reagents must be butyltin free. Grignard reagents are tested to insure they are of acceptable reactivity and do not contain butyltins.

The reproducibility of the procedure is monitored by analyzing duplicates of randomly chosen samples. Acceptance criteria for duplicates with concentrations greater than 10 times the MDL is ± 20 percent difference.

Accuracy is determined by analyzing a certified reference material. The butyltin concentrations for the certified reference material must be $\pm 20\%$ of the certified concentration range for tetrabutyltin, tributyltin, dibutyltin, and monobutyltin. The acceptable range for the spike blank percent recoveries is 70-130% for tetrabutyltin, tributyltin, and dibutyltin and 40-100% for monobutyltin. GC-FPD precision is controlled by running calibration solutions within every run.

4.1.3.2. Inorganic Contaminants

Blank analyses. One blank analysis is run with every set of samples (20 samples/set). Blanks follow the exact procedure of regular samples. Blanks must be

less than three times the Limit of Quantitative Detection (LQD) and/or less than 20% of the sample analyte value.

Extraction efficiencies. Extraction efficiencies are monitored by comparison of the % recoveries for Standard Reference Materials (SRM) that are run with each batch of samples. The SRMs used for sediments are NIST 1646, MESS-2, and PACS-1. The SRM used for tissues is NIST 1566 oyster tissue. Two SRM samples are run with each set of 20 samples. The recoveries must be in the range of 75–125% of the average percent recovery historically obtained by that digestion method. The extraction efficiency of the ICP is monitored by spike adds. In addition, one pre-extraction spike will be done with each batch of 20 samples and recoveries must be in the 50-150% range.

Instrument variability. Instrument variability is determined by replicate analyses of standard mixtures and samples. Values for all analytes range between 1 and 10% RSD for the standards and samples. Overall analytical precision is determined by processing of replicate samples. A replicate is run with every ten samples. The relative percent difference is usually less than 15% for analytes whose concentration are 10 x Method Limit of Quantitation.

Analytical accuracy Analytical accuracy is determined by the analysis of the SRM materials (sediments NIST 1646, PACS-1, MESS-2, and oyster tissue NIST 1566). Past accuracy has been $\pm 25\%$ of the certified concentration range for 90% of the analytes.

In summary, the frequency of URI QC procedures for inorganic analyses is as follows:

- o Blanks - 2 every 20 samples
- o Spike Additions - 1 every 20 samples
- o Duplicates - 1 every 10 samples
- o SRMs -1 every 10 samples.

Thus, a minimum of seven QC samples are analyzed for every 20 field samples processed. Corrective action is immediately taken if QC values fall outside of defined limits. These actions may include re-injection, re-extraction, preparation of new standard solutions, replacing reagents, and repair of instruments.

4.2 TOXICITY TEST PROCEDURES

4.2.1. Amphipod Test Procedures

Standard Operating Procedures and QA/QC Procedures for conduct of the 10-day, solid-phase test with *Ampelisca abdita* are summarized in Appendix C. Amphipods will be exposed to test sediments for 10 days with five replicates under static conditions

using 30 ppt filtered seawater. Exposure chambers will consist of quart-sized canning jars with an inverted glass dish as a cover. Two hundred milliliters of control or test sediment will be placed in the bottom of the jar and covered with approximately 600 ml of water. Air will be delivered via air pumps into the water column by a polystyrene 1-ml pipette inserted through a hole in the cover to ensure acceptable dissolved oxygen concentrations (>60% saturation). To stabilize the temperature, tests will be conducted in a water bath and lighting will be continuous during the 10-day test to inhibit swimming behavior of the organisms.

At the beginning of each test, twenty animals for each species will be distributed randomly into 100-ml plastic beakers containing the appropriate water for that species. The beakers will be examined for dead or outsized animals, which will be replaced. The beakers will be randomized, air delivery to the exposure containers stopped, and the amphipods and mysids added to their respective test chambers. For amphipods, after one hour, nonburrowing animals will be replaced, and air delivery will be restarted, initiating the test. *Ampelisca abdita* will not be fed during the test. All observations conducted during the test and at completion will be performed "blind" to ensure that observer bias will not effect results. Exposure containers will be checked daily and the number of individuals that are dead, moribund, on the sediment surface, and on the water surface will be recorded. Salinity (refractometer), dissolved oxygen (Orion model 970800), and pH (Orion model 250A) will be monitored twice during the test (generally on days 2 and 7).

The primary response criteria to indicate toxicity of test sediments in these assays will be survival. After 10 days, the assay will be terminated and the contents of each exposure chamber sieved through a 0.5-mm mesh screen. The material retained on the sieve will be sorted under a stereomicroscope and the recovered animals counted. Any missing individuals will be assumed to have died and decomposed during the test and will be counted as dead. "Live-picking" of the samples at the termination of the tests will be the preferred method of analysis. However, if problems develop in the laboratory, and live-picking will not be possible, the sample material will be sieved and preserved in 5% buffered formalin with Rose Bengal stain for later examination. Statistical significance will be calculated for each sample with a mean survival less than that of its respective performance control. Survival data from these samples will be compared to that of the control with a one-way, un-paired, *t*-test that assumes unequal variance. The alpha level will be 5%.

4.2.2. Sperm-Cell Test Procedures

Test procedures will follow EPA's protocol for complex effluents (EPA, 1988--Standard Operating Procedure; Appendix C). Female sea urchins will be transferred to a large Carolina dish filled with enough seawater to just cover their shells. Eggs will be obtained from the females by electrical stimulation. The electrodes from a 12-volt transformer will be gently placed on the shell close to the gonadopores for about 30 seconds. The red eggs, which pool above the gonadopores, will be collected with a 10

ml disposable syringe with a blunted large-gauge needle. Eggs will be collected from all females and kept at room temperature (about 20 °C) until use (no longer than two hours). The final egg concentration in the stock solution will be 2,000 per ml. Sperm also will be collected from male sea urchins by electrostimulation, using a 2 ml syringe, and kept on ice. The time of sperm collection will be recorded; the sperm will be exposed to the test material within one hour after collection. The sperm will be diluted to a stock concentration of 50 million per ml, and the stock kept on ice until use.

A test begins with the introduction of 100 µl of well-mixed sperm stock to each test and control vial (which contained 5 ml of sample or control seawater) so that there will be about 5 million sperm in each vial. The test vials then will be covered, the time will be recorded and the vials held at 20 to 22 °C for one hour. Both natural seawater and brine controls will be used when the samples required salinity adjustments. After one hour, the egg stock will be mixed by gentle aeration and 1 ml of stock added to each exposure vial. The vials will be gently swirled to insure mixing and covered again. After an additional 20 minutes, 1 ml of 10% buffered formalin in seawater will be added, terminating the test. The fertilization will be evaluated by examining 100 individual embryos from each replicate with a compound microscope at 100X magnification. A reference toxicant test will be performed using sodium dodecyl sulfate.

Statistical significance will be calculated for each sample with a mean fertilization rate less than that of the performance control. Fertilization data from these samples will be compared to that of the control with a one-way, un-paired, *t*-test that assumes unequal variance. The alpha level will be 5%.

5.0 SAMPLE AND DATA MANAGEMENT PROCEDURES

5.1 SAMPLE CUSTODY PROCEDURES

All samples, whether generated in the laboratory or field, will receive a unique sample number generated and tracked by the QA/QC Officer. This number will be affixed to sampling containers, and pertinent collection information (station, date, time, depth, etc.) will be entered into sampling logs by field personnel. This information will be transferred to data bases as described in the draft Data Management Plan (Appendix D). Duplicate logs will be maintained to minimize loss of information.

Because different individuals may be involved in the collection and distribution of individual samples, chain-of-custody forms (Figure 5-1) will be maintained for each set of samples transferred. These forms will follow each set of samples from collection through sample archiving. Duplicate forms will be maintained to minimize loss of information. Sample condition will be evaluated by the appropriate project staff at time of receipt from field collection personnel.

Samples for physical and biological analysis will be stored prior to analysis as described in relevant SOPs (Appendix B; Mueller *et al.* 1992). Samples for chemical analysis will be stored on ice during transport to the laboratory (URI) and frozen at -20°C (sediments and tissues) or refrigerated at 1-5°C in the dark (water samples) prior to shipment.

5.2 DOCUMENTATION, DATA REDUCTION AND REPORTING

Toxicological/biological assessment data collected by SAIC personnel will be entered directly onto test-specific standardized data sheets and log books. Examples of raw data sheets are provided in Appendix B. Duplicate copies will be maintained to minimize loss of information. The raw data also will be entered into computerized data bases (as described in the draft Data Management Plan).

Test-specific data reduction practices and statistical analyses for toxicity data are described in Appendix B. Generally, data reduction and statistical analyses will be performed by computerized utilities (e.g., Statistical Analysis System, SAS). Statistical analysis approaches will be reviewed and approved by a professional statistician with SAIC.

Final data reports will be prepared for the project Principal Investigators upon completion of biological assessment data validation and analysis. These reports will contain descriptions of test conditions, results, and ancillary observations, and may contain preliminary interpretations. Additionally, raw data will be accessible through centralized data bases and data sheets.

5.3 DATA VALIDATION

Data validation conducted under this Work Plan will conform to NEESA Level C analytical data validation practices. For all chemistry results, the data will be subjected to EPA Tier II validation procedures (USEPA, 1993). All raw and computerized data reported in this project will be subjected to a 100% review by the personnel responsible for each assessment. An additional review will be performed by a second individual to identify errors in recording, transcription, and reporting. Raw data sheets and laboratory notebooks will be reviewed in this process. Data that do not meet the standards described in this document will be reported with an explanatory notation. The Principal Investigators will make the final determination as to data validity. Descriptions of interpretation and synthesis activities utilizing suspect data will be prefaced with an explanation of data quality.

5.4 PERFORMANCE AND SYSTEMS AUDITS/CORRECTIVE ACTION

Audits of all project activities may be performed by the Quality Assurance

Officers at any time over the course of this project. Such audits will compare QA/QC activities actually performed with those identified in this W/QAPjP. These audits may involve site visits, direct observations of technical performance, review of all levels of data documentation, review of field logs and chain-of-custody procedures, and QA/QC review of reports and products. Full cooperation will be extended by project staff to either QA Officer during such audits to facilitate identification of non-compliance and to enhance the quality of data generated through conduct of work assignment activities. Results of audits will be reported to the Project Manager. After consultation, the Project Manager will develop and implement responses appropriate to audit findings.

The QA Officer also will assess performance through site visits during field sampling and laboratory analysis activities. This individual will have the authority to modify activities to bring them into conformance with quality control requirements. In the event of continued non-conformance, work associated with the specific activity will be stopped until the appropriate corrective action is implemented.

Corrective action in this project has two components: technical and managerial. Technical correction action involves steps taken to rectify isolated problems associated with performance of specific project activities. Failure to meet minor procedural requirements will be brought to the attention of the Principal Investigator associated with those activities by the technical personnel involved. Using professional judgement on an individual incident basis, a decision will be made as to how to report the infraction and its significance. More serious infractions, such as those involving data acceptability criteria as described above, may result in rejection of an entire data set. In consultation with the Project Manager, and with consideration of project resources, a decision may be made to repeat the activity following correction of performance deficiencies. All technical correction actions will be documented fully in subsequent reports.

Managerial corrective action involves steps taken to rectify repeated performance issues arising from continued failure to meet project objectives. Such problems may be due to inappropriate activity methodologies, inappropriate staffing assignments, or other causes. The Principal Investigators will jointly assess activity methodologies to identify alternate approaches or modification of project objectives. Decisions will be communicated to the HNUS Project Manager and the TRC as appropriate. Staff supervisors and subcontract Project Managers will address problems of performance following the governing employment and contractual agreements.

5.5 CALIBRATION PROCEDURES AND PREVENTIVE MAINTENANCE

Instruments must be calibrated prior to analysis, after each major equipment disruption, or whenever ongoing calibration checks do not meet recommended control limit criteria. All calibration standards used should be traceable to recognized

organizations for the preparation of QA/QC materials. Detailed calibration procedures for chemical analytical instrumentation are contained in Appendix B.

A variety of physical measurements are made during each solid-phase toxicity test procedure. These include pH, salinity, dissolved oxygen, and temperature. The method used, the level of precision, and the frequency and method of calibration are listed in appropriate SOPs. The frequency of measurement is test specific, as is the type of biological data monitored, and also is detailed in the SOPs for the various tests. SOPs contained in Appendix B describe the methods used for calibrating and maintaining all essential equipment to be used in this project.

6.0 REPORT PREPARATION

Three general types of products will be generated as part of site-specific ecological risk assessments at Navy sites: site-specific addenda, individual data products, and the ecological risk assessment reports. The form and intended use of each of these products is discussed below.

6.1 SITE-SPECIFIC ADDENDA

Site-specific addenda to the master Work Plan will be developed for each Navy site under investigation in this program. The format of these addenda is shown in Table 6-1. Each addendum will contain plans for the risk assessment activities to be conducted at that site. In addition to summarizing background information relevant to the site, these plans will identify the objectives and scope of each assessment, and will describe the site-specific application of the approaches to Problem Formulation, Analysis, and Risk Characterization described in Section 2.0 of the master Work Plan. Perhaps most importantly, these addenda will present site-specific conceptual models of the ecological risks posed by each Navy disposal site. These models will serve to summarize current understanding of the CoCs and hypothesized exposure pathways, and will identify the ecological systems/species/receptors of concern in Narragansett Bay and the measurement endpoints to be employed to evaluate potential adverse ecological effects. Site-specific addenda also will include descriptions of individual project organization and schedules.

The information contained in site-specific addenda will be used by the regulatory community to evaluate the appropriateness, completeness, and timeliness of proposed assessment activities. Addenda will be submitted for review in draft for comment per FFA requirements. Upon incorporation of regulatory comment, these documents will be submitted in final form to the administrative record.

6.2 SITE-SPECIFIC DATA PRODUCTS

Several types of data products may be generated during the course of site-specific investigations. Examples of these include:

- preliminary evaluations of CoC concentration in various environmental exposure media
- interim summaries of exposure and ecological effects data
- results of QA/QC evaluations
- reports of special investigations, such as analysis of landfill toe migration through interpretation of aerial photographs, or of sediment geotechnical properties and distribution

The information contained in site-specific data products may be conveyed as deliverable documents or orally at Ecorisk Advisory Group meetings, or Technical Review Committee (TRC) meetings. This information can be used by the regulatory community to evaluate preliminary investigation findings, and may be used to redirect subsequent project activities to ensure the attainment of project objectives.

6.3 SITE-SPECIFIC ECOLOGICAL RISK ASSESSMENT REPORT

The site-specific ecological risk assessment report will document project activities and findings in a manner which satisfies the objectives established for each project. The format of this document is shown in Table 6-2. It's structure conforms to guidance provided by U.S. EPA (1989b, 1992a, b) and the Narragansett Bay Ecorisk Advisory Group.

Section 1.0 of this document will provide an executive summary of the background, objectives, scope, and major findings of the investigation. It will be written in a manner easily understood by the general public to support communication of study results.

Section 2.0 will provide introductory background material concerning the site, and will lay out the purpose, objectives, and scope of the investigation. This section will establish the context within which the investigation was conducted.

Section 3.0 will describe application of the approach to site-specific Problem Formulation described in Section 2.1 of this master Work Plan. It will include a detailed site characterization, will identify the assessment and measurement endpoints (including CoCs and ecological systems/species/receptors of concern), and will present the site-specific conceptual model.

Section 4.0 will describe application of the approach to site-specific Exposure Assessment described in Section 2.2 of this master Work Plan, and will present the findings of this activity. It will include discussion of sources and exposure pathways, analyses of fate and transport, and estimates of CoC exposure point concentrations. Important findings of special investigations conducted to evaluate exposure issues also will be presented in this section. A discussion of the uncertainties associated with Exposure Assessment will be included.

Section 5.0 will describe application of the approach to site-specific Ecological Effects Assessment described in Section 2.3 of this master Work Plan, and will present the findings of this activity. It will include discussion of the known effects of the CoCs, and the results of site-specific toxicity and ecological effects investigations. Existing toxicity-based criteria and standards specific to each exposure medium will be identified. Important findings of special investigations conducted to evaluate adverse ecological effects also will be presented in this section. A discussion of the uncertainties associated with Ecological Effects Assessment will be included.

Section 6.0 will describe application of the approach to site-specific Risk Characterization described in Section 2.4 of this master Work Plan, and will present the findings of this activity. It will include evaluation of the weight-of-evidence linking site-specific CoCs to adverse ecological effects, using all information available. Results of approaches used to quantify ecological risks (such as simulation modeling and joint probability analysis) will be included as appropriate. It also will incorporate discussions of the uncertainties associated with Risk Characterization activities and conclusions.

If appropriate, a seventh section may be included which addresses specific ecological risk issues important to the Navy, U.S. EPA, the State of Rhode Island, Natural Resource Trustees, and/or the public. Examples of such issues include the severity and significance of ecological risks to Narragansett Bay, and other potential sources of stress to ecological systems at each site. Discussion will be held with the Narragansett Bay Ecorisk Advisory Group to identify issues for inclusion in this section.

Literature sources cited in the ecological risk assessment report will be listed in Section 8.0. Data and other appendices will be attached to the document as appropriate.

The information contained in site-specific ecological risk assessment reports will be used by the regulatory community to evaluate the ecological risks to Narragansett Bay posed by individual Navy sites, and to select appropriate remedial options. Ecological risk assessment reports will be submitted for review in draft and draft final versions. Upon incorporation of regulatory comment, these documents will be submitted in final form to the administrative record.

7.0 HEALTH AND SAFETY

All project personnel will comply with the Health and Safety policies and protocols detailed in Appendix E. All sampling personnel will be provided adequate training for handling of potentially hazardous materials such as decontaminating fluids or sample preservatives as required by SARA. Sampling and other data gathering work performed from any size boat or vessel will be supervised by trained boat handlers/licenced captain. This individual will assume authority over all personnel in any situation where safety is deemed in question.. In addition, OSHA 40-hour hazardous waste site worker training will be provided to any individual required to conduct sampling within the boundaries of uncontrolled hazardous waste sites as defined in OSHA 29CFR 1910.120..

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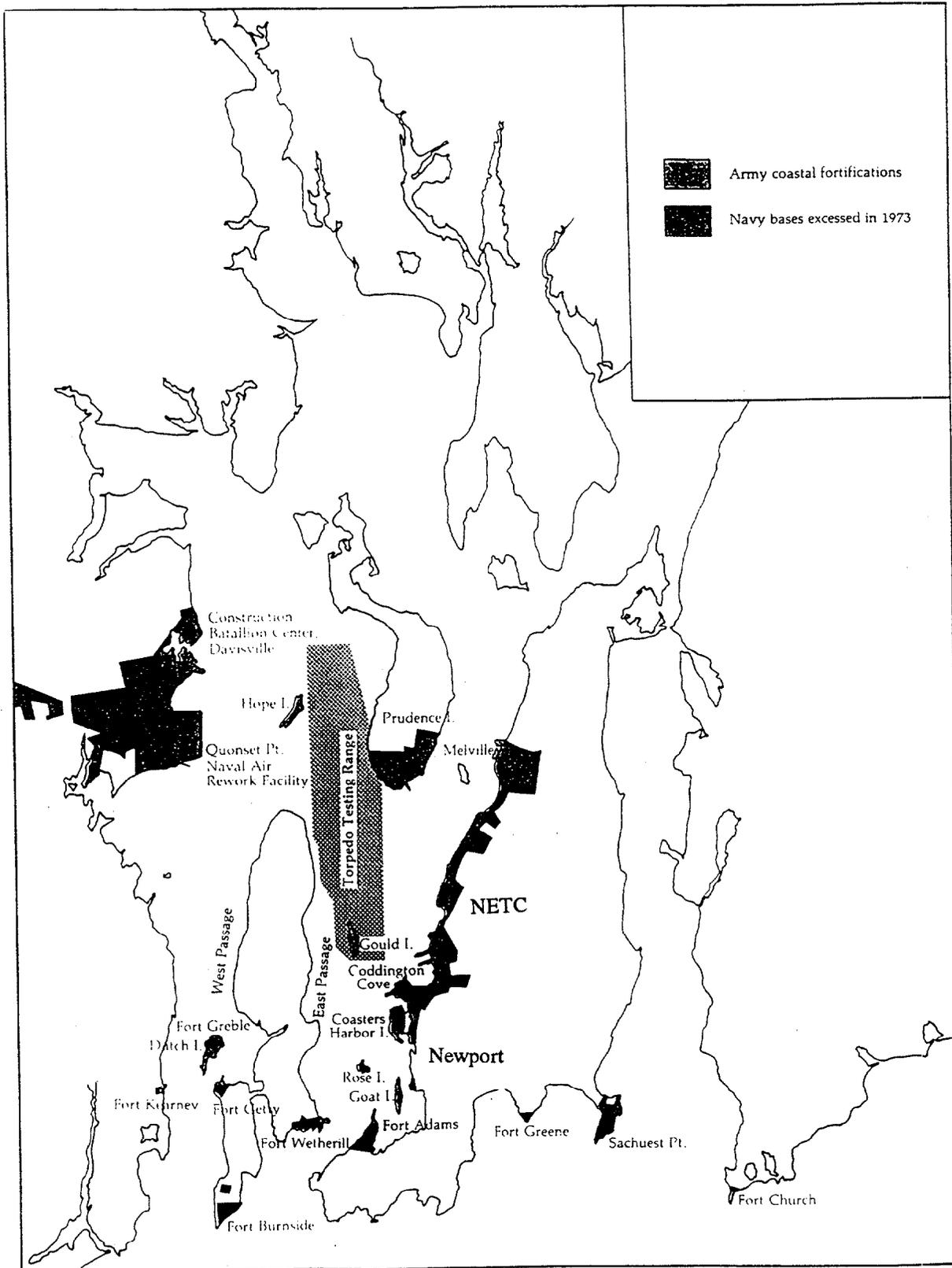


FIGURE 1-1. NAVY BASES AND COASTAL FORTIFICATIONS IN NARRAGANSETT BAY (FROM OLSEN ET AL. 1980).

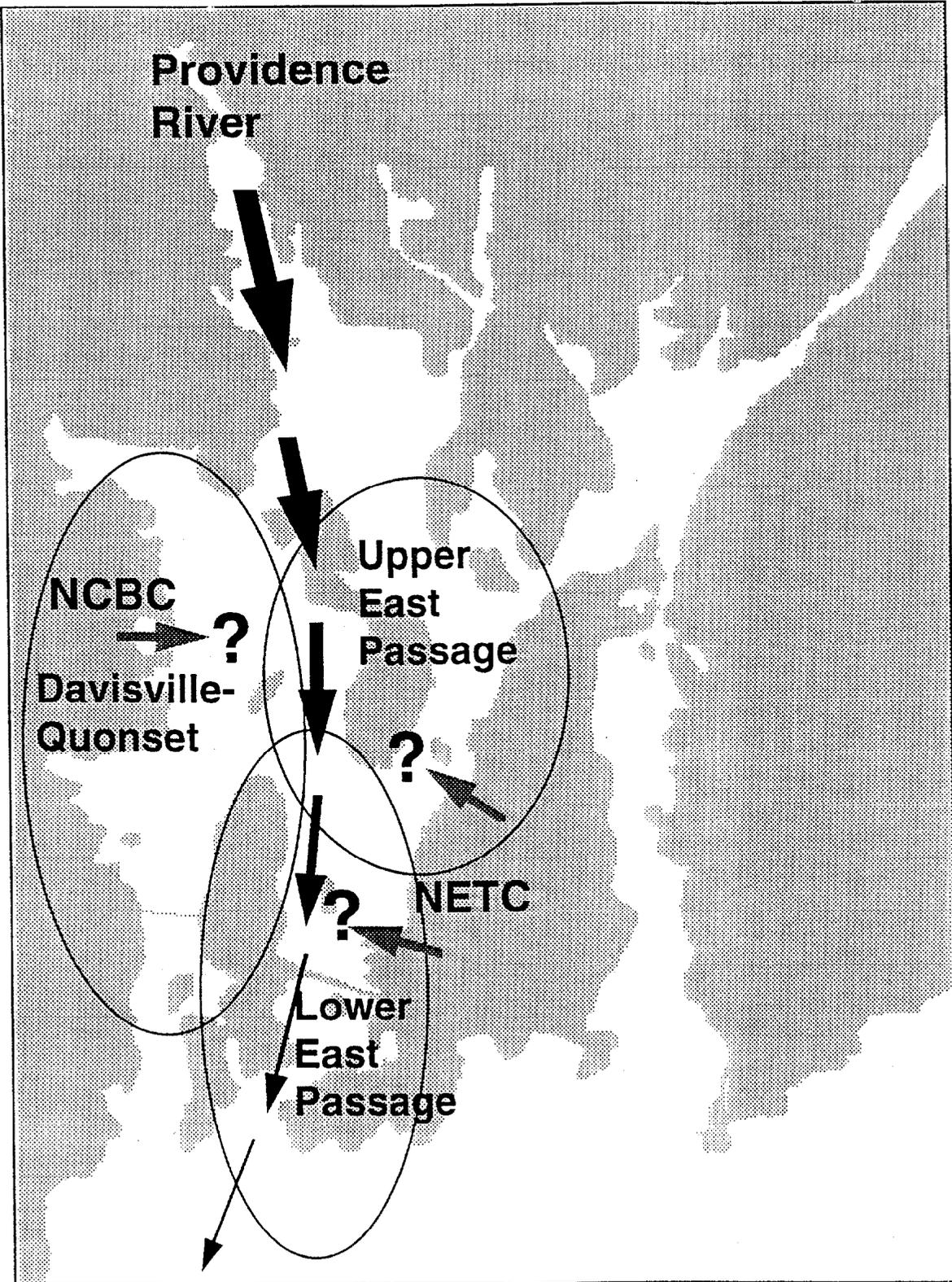


FIGURE 1-2. RELATIONSHIP BETWEEN NAVAL FACILITIES, STUDY AREAS, AND THE DOWN-BAY GRADIENT OF CONTAMINATION.

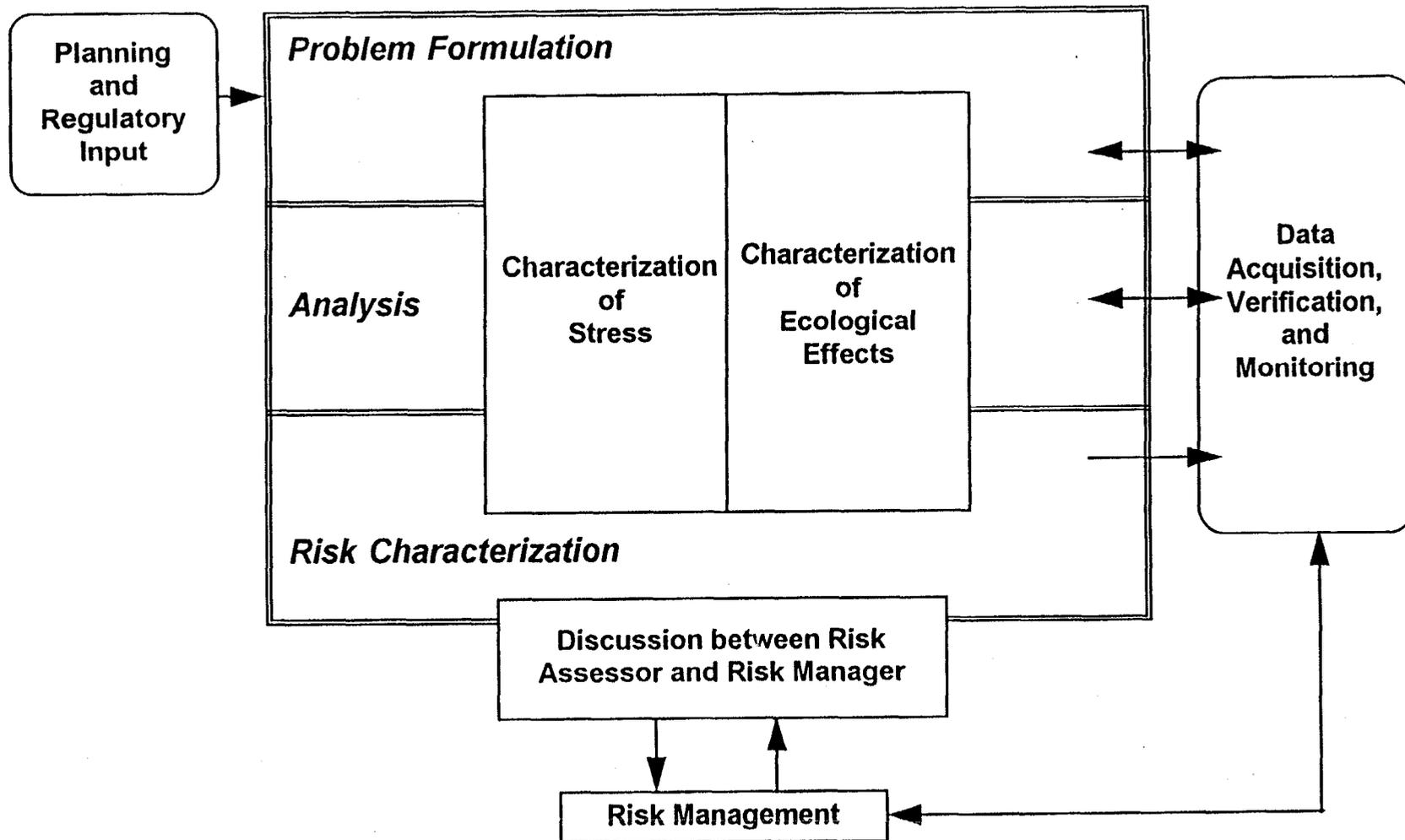


FIGURE 2-1. EPA'S FRAMEWORK FOR ECOLOGICAL RISK ASSESSMENT.

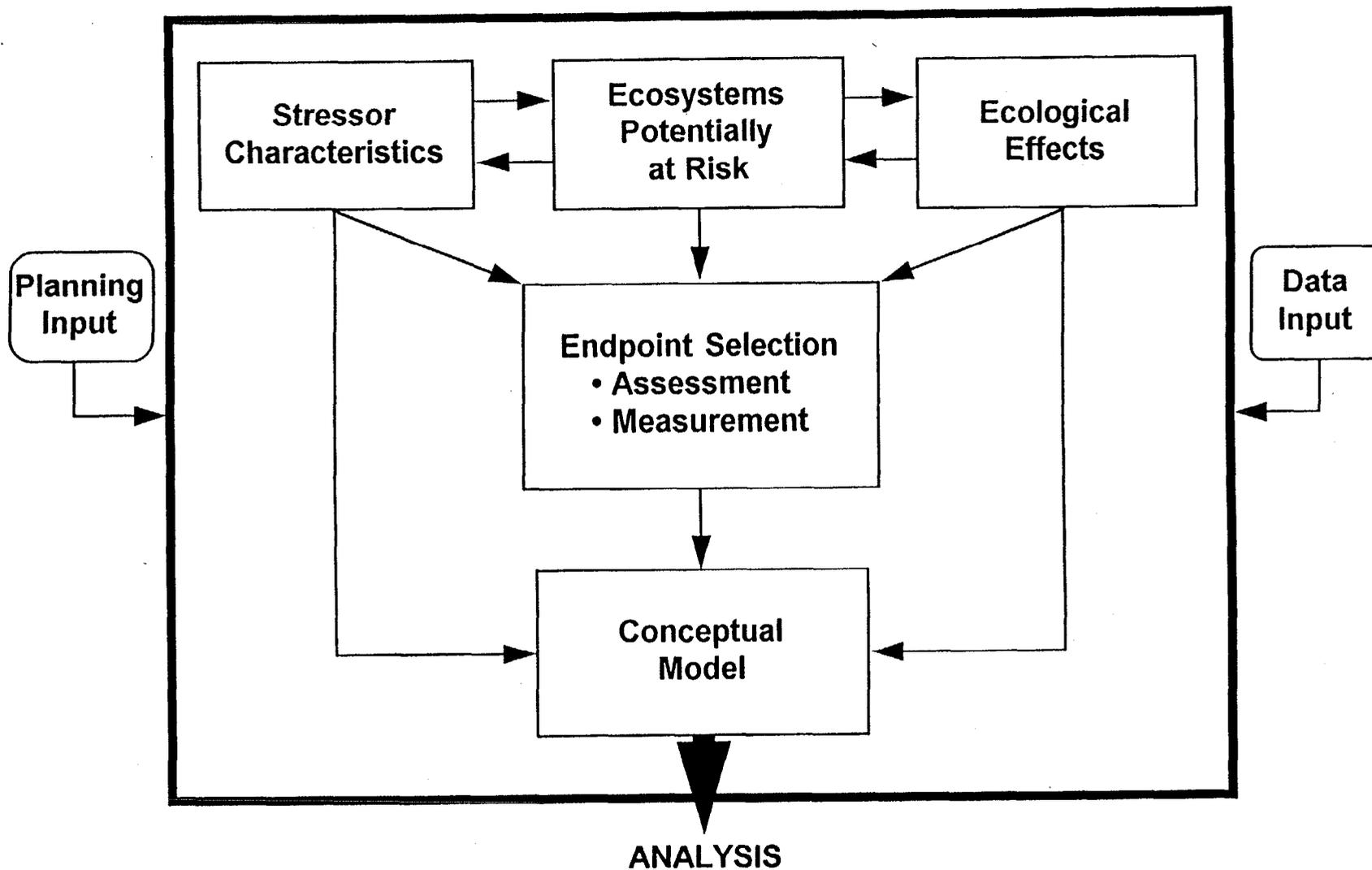


FIGURE 2-2. PROBLEM FORMULATION IN ECOLOGICAL RISK ASSESSMENT.

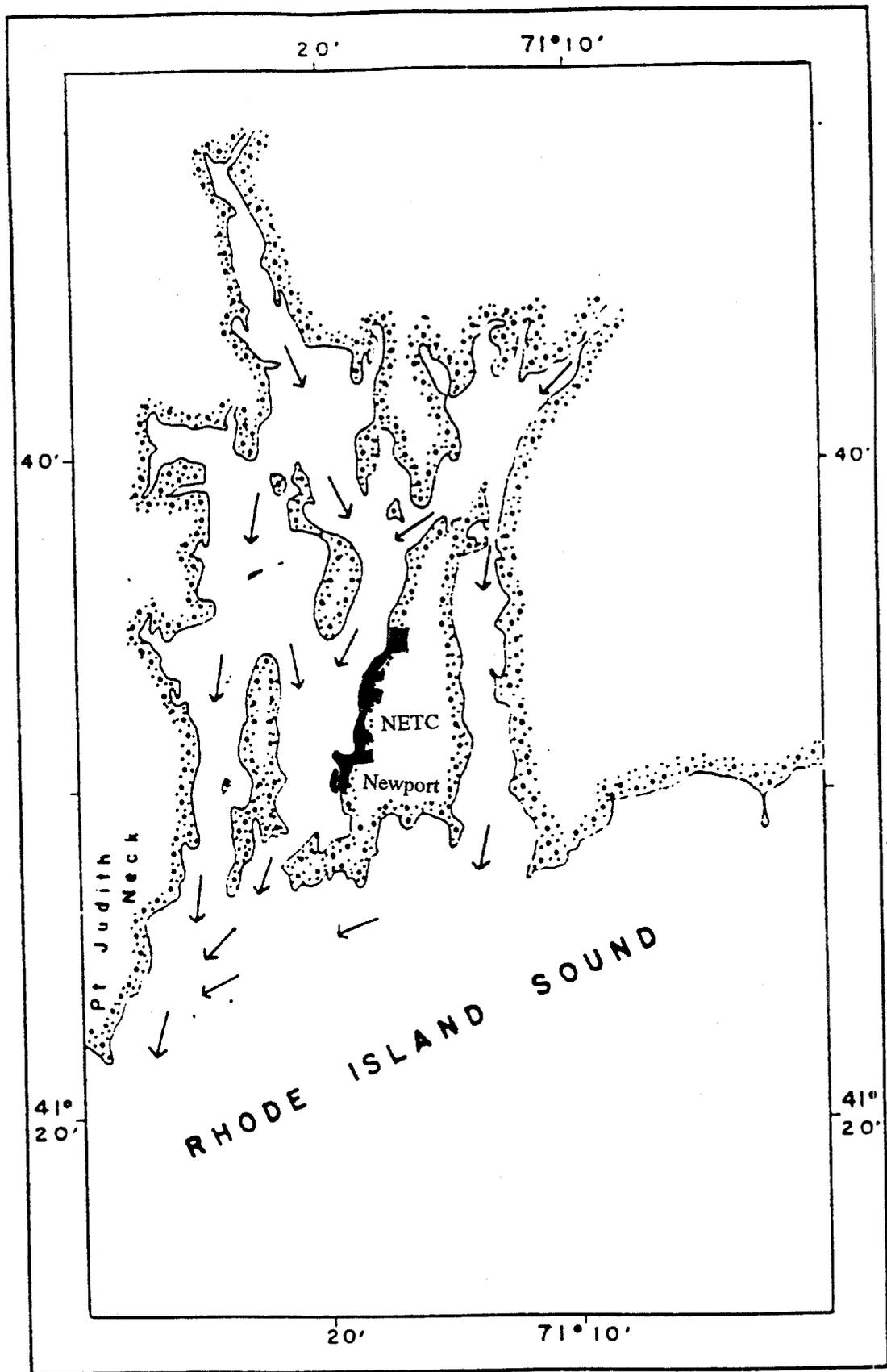


FIGURE 2-3. NON-TIDAL SURFACE CURRENT PATTERN FOR THE NARRAGANSETT BAY SYSTEM (FROM MCMASTER 1960; AFTER HICKS 1959).

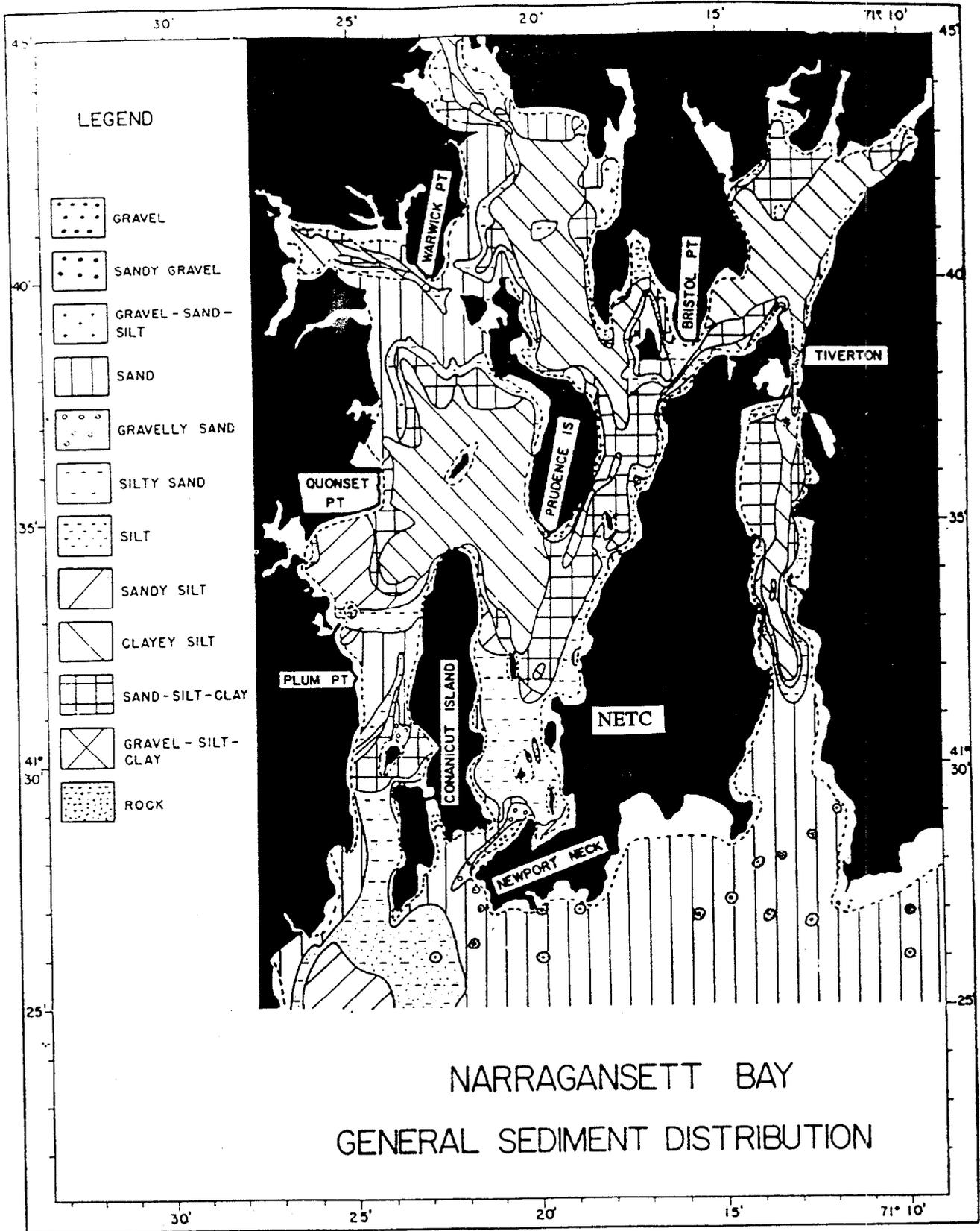


FIGURE 2-4. SEDIMENT DISTRIBUTION IN THE NARRAGANSETT BAY SYSTEM BASED ON GRAVEL, SAND, SILT, AND CLAY CONTENT (FROM MCMASTER 1960).

HABITATS OF NARRAGANSETT BAY

(Adapted from the original by Applied Science Associates, 1992)

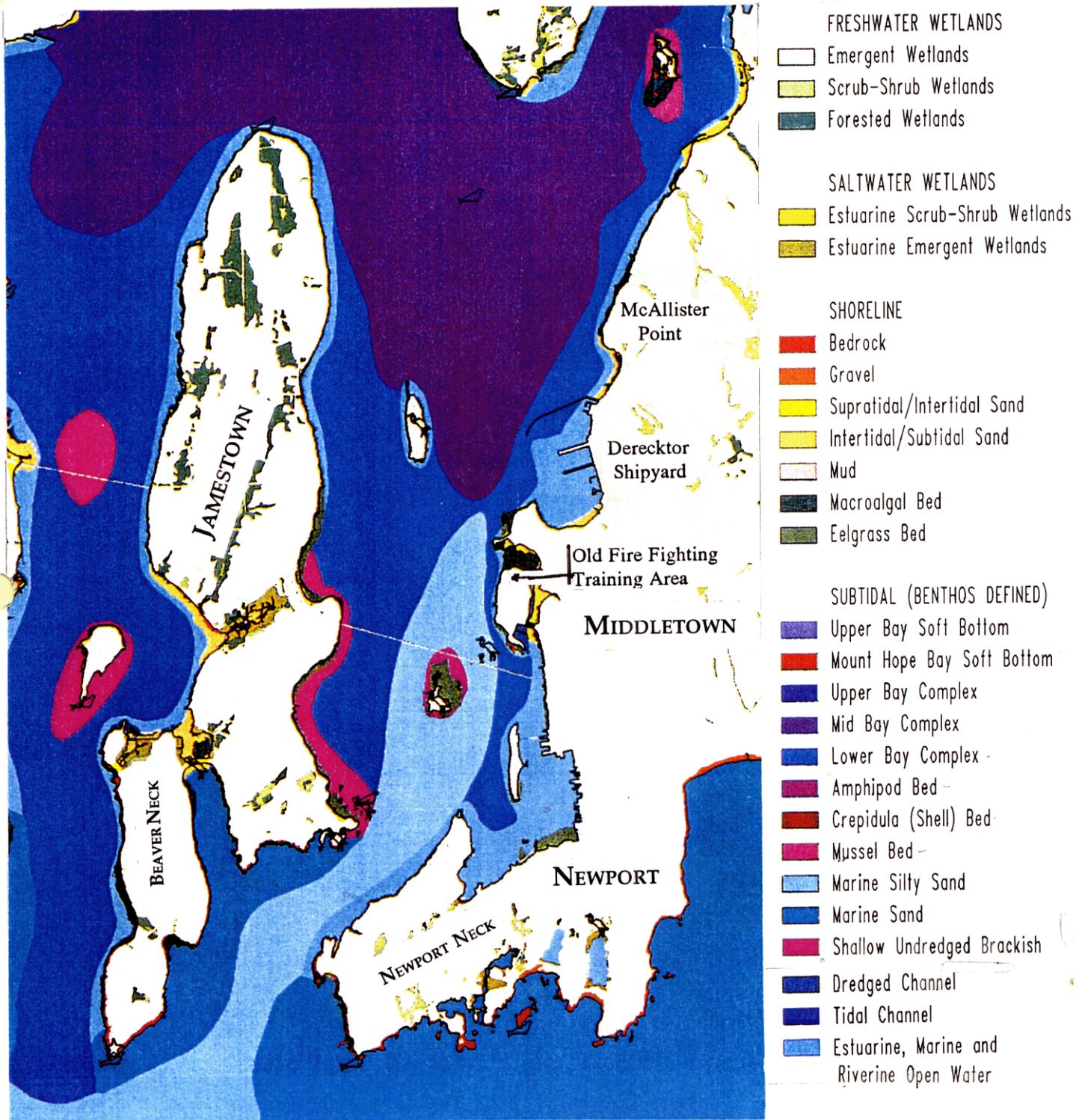
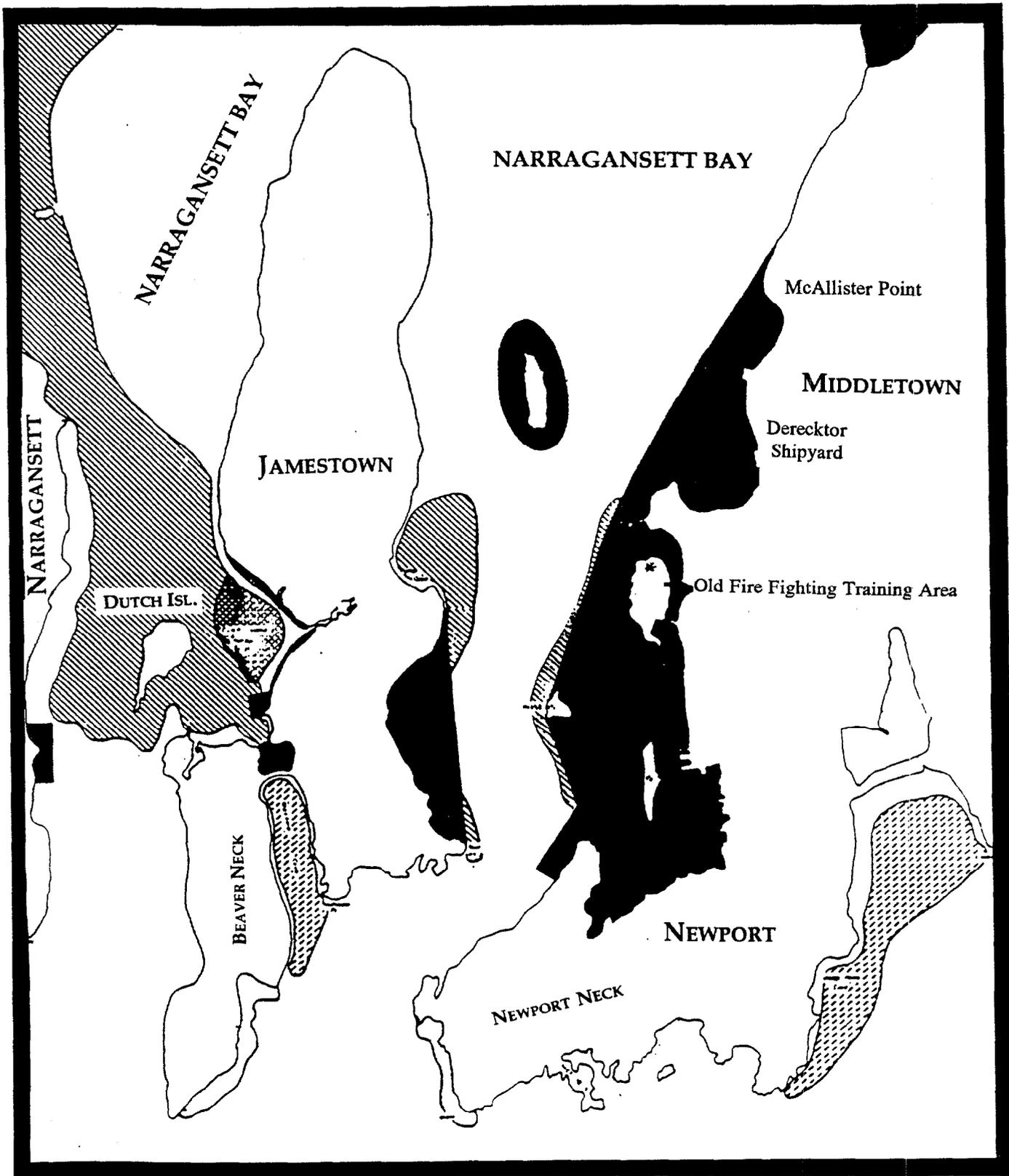


FIGURE 2-5. SUBTIDAL BENTHIC HABITATS OF NARRAGANSETT BAY (FROM FRENCH ET AL. 1992A). SEE TABLE A2-1 FOR DESCRIPTIONS OF HABITAT TYPES.

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- | | | |
|---|--|--|
|  Hard Clam
(<i>Mercenaria mercenaria</i>) |  Eastern Oyster
(<i>Crassostrea virginica</i>) |  Closed for shell fishing |
|  Surf Clam
(<i>Spisula solidissima</i>) |  Mussel, salt water
(<i>Mytilus edulis</i>) | |

FIGURE 2-6. SHELLFISH AREAS OF LOWER EAST AND WEST PASSAGES IN NARRAGANSETT BAY (FROM PRATT ET AL. 1992).

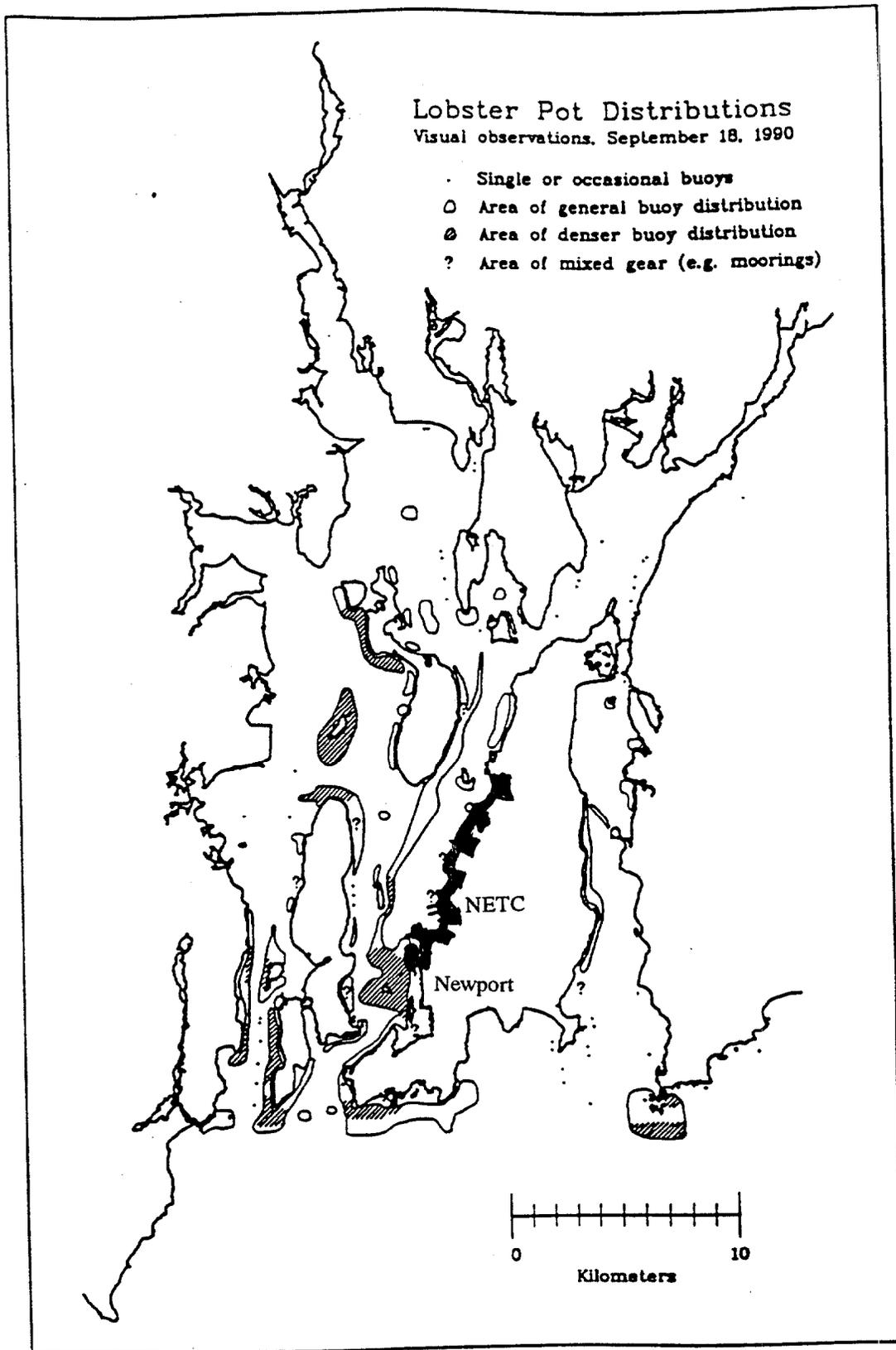


FIGURE 2-7. DISTRIBUTION OF LOBSTER POTS FROM VISUAL BUOY OBSERVATIONS, 18 SEPTEMBER 1990 (FROM FRENCH ET AL. 1992C).

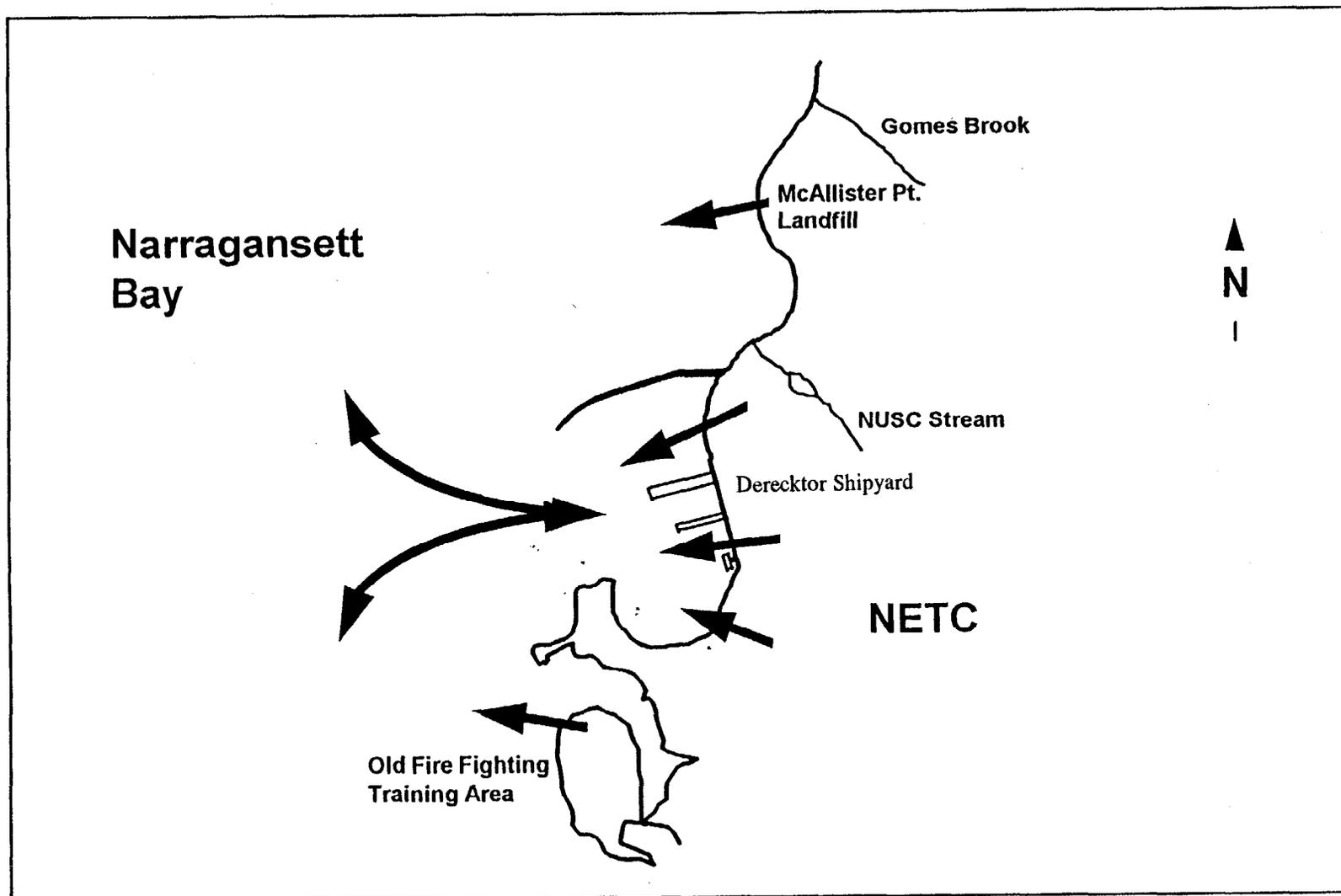


FIGURE 2-8. SECOND TIER CONCEPTUAL MODEL OF CONTAMINANT MOVEMENT.

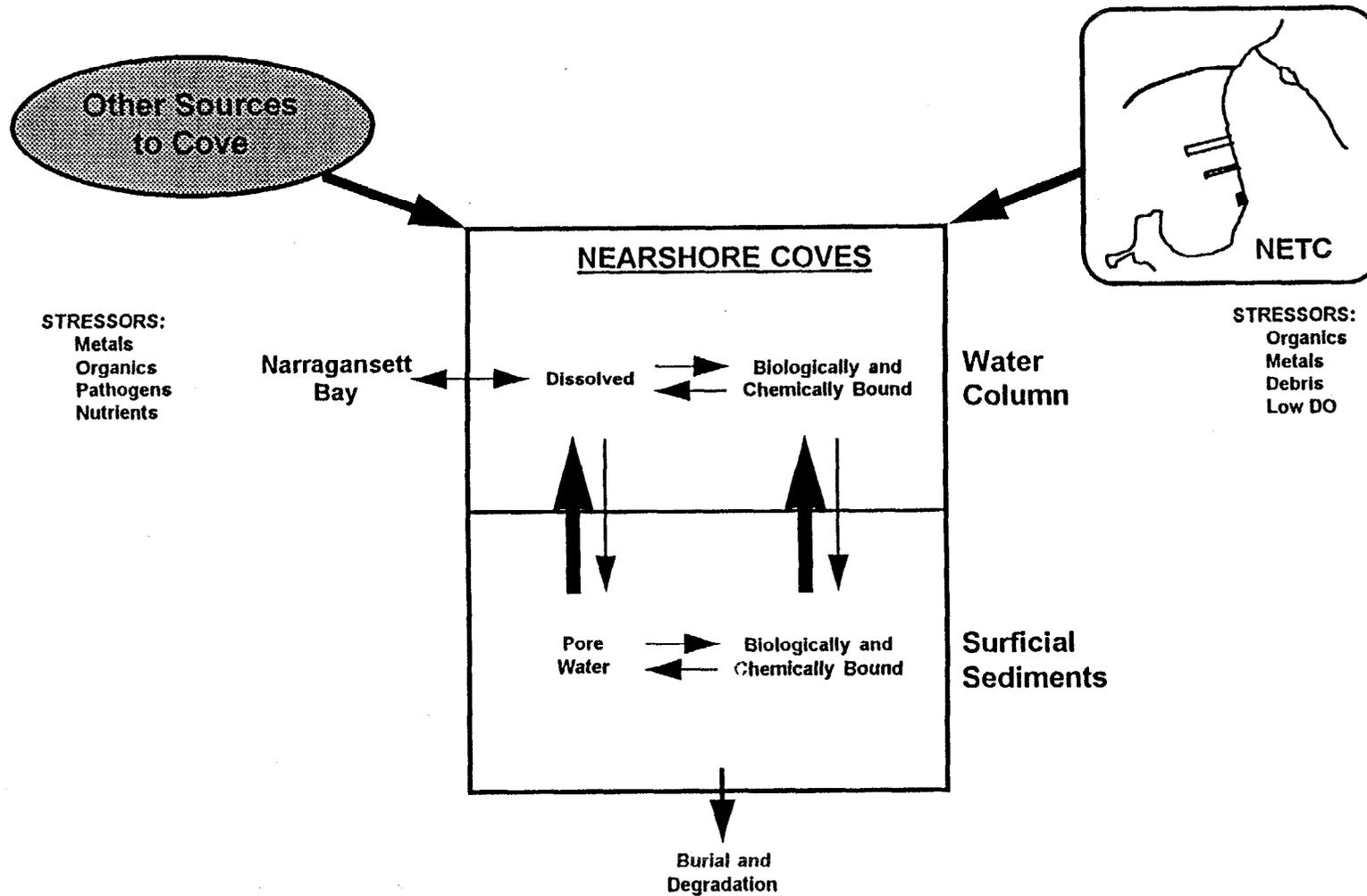


FIGURE 2-9. THIRD TIER CONCEPTUAL MODEL OF CONTAMINANT BEHAVIOR IN NEARSHORE COVES.

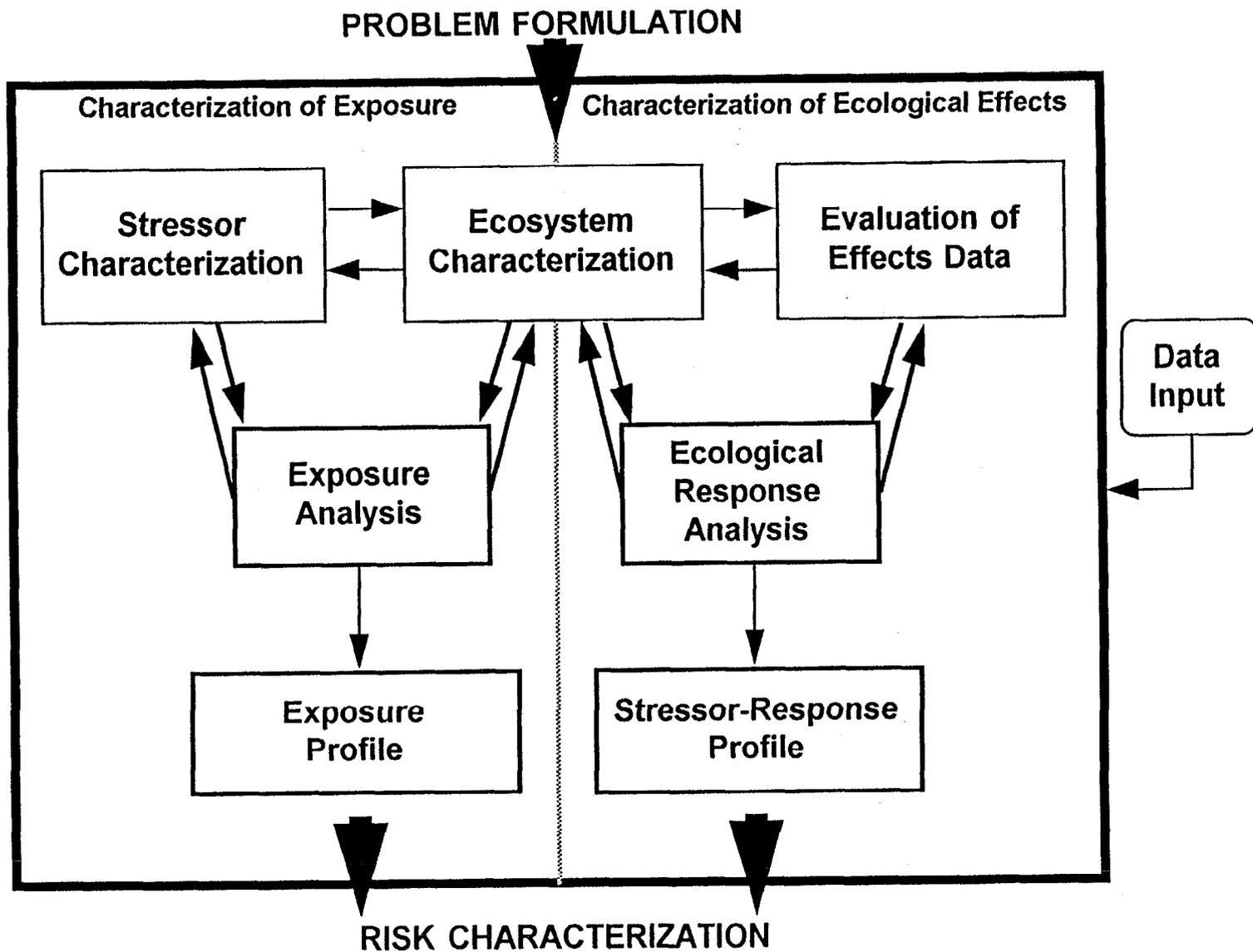


FIGURE 2-10. ANALYSIS IN ECOLOGICAL RISK ASSESSMENT

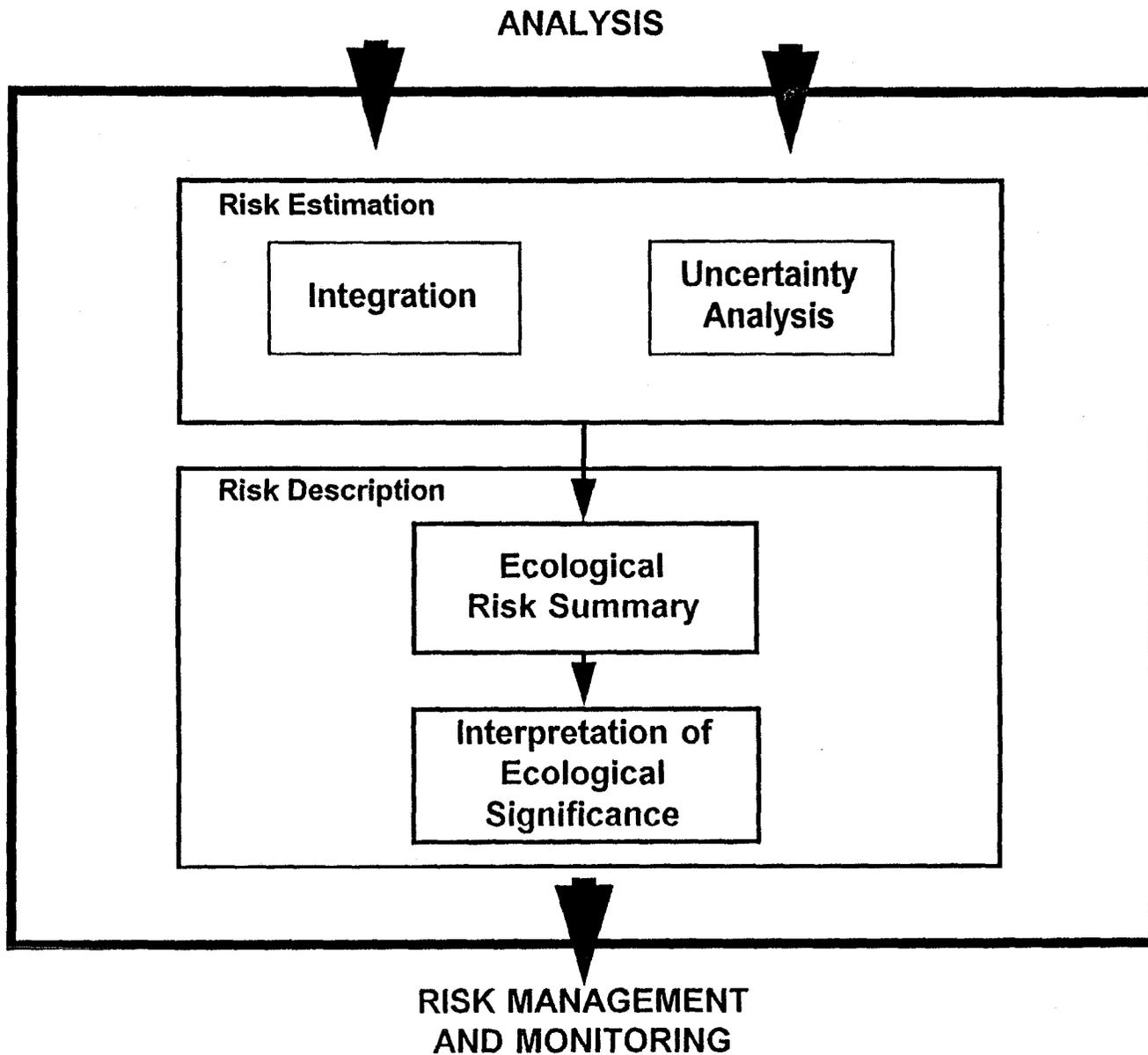


FIGURE 2-11. RISK CHARACTERIZATION IN ECOLOGICAL RISK ASSESSMENT

TABLE 2-1. Key to sub-tidal benthic habitat types indicated in Figure 2-5 (from French et al. 1992a).

Code	Habitat Description
Marine Silty Sand	This is a silty sand habitat typical of Rhode Island Sound and extending up into the East Passage. The fauna are characterized by marine species such as <i>Astarte</i> , <i>Cyclocardium</i> , <i>Byblis serrata</i> and <i>Arctica islandica</i> .
Marine Sand	This habitat is found at the mouths of the bay and in RI Sound. It is characterized by fine sands with marine species such as <i>Spisula</i> , <i>Echinarachnius</i> , and <i>Spiophanes bombyx</i> .
Lower Bay Complex	This is a lower bay complex on a variety of mixed sediments containing sand. <i>Mytilus</i> and <i>Crepidula</i> shells may be locally abundant. The mid-estuarine and estuarine-offshore species found here include <i>Pherusa affinis</i> , <i>Aricidea</i> , and <i>Ampelisca vadorum</i> .
Mid Bay Complex	This habitat is found in the deeper waters of the mid-bay and the channels of Mt. Hope Bay and the upper bay on clayey silt and sand-silt-clay. The fauna are mid-estuarine and estuarine-offshore, including <i>Mulinia</i> , <i>Mediomnastus</i> , <i>Nucula annulata</i> , <i>Nephtys</i> , and <i>Yoldia</i> .
Mussel Beds	Mussel beds (<i>Mytilus edulis</i>).
Crepidula Beds	Crepidula beds.
Amphipod bed	Areas dominated by tubes of <i>Ampelisca abdita</i> .

TABLE 2-2. Concentrations of organic contaminants and cadmium in sediments and mussel tissue (Water Quality Laboratory Report, U.S. Army Corps of Engineers, 1988).

	PCBs	PHCs	Cadmium
Gould Island			
Sediment	0.02-0.30 ppm	100-300 ppm	N.D. (<2 ppm)-15.0 ppm
Tissue	0.04-0.36 ppm	not measured	N.D. (<0.4 ppm)
McAllister Point			
Sediment	0.01-2.03 ppm	30-1,100 ppm	N.D.(<2 ppm)-12.0 ppm
Tissue	0.01-0.30 ppm	not measured	N.D. (<0.4 ppm)

N.D. = not detected

TABLE 2-3. Concentrations of organic contaminants in surface sediments from the East Passage of Narragansett Bay (Quinn *et al.* 1992).

Station	Σ PHCs (ppm)	Σ PCBs (ppb)	Σ PAHs (ppm)
12 (E. Mid Prudence Is.)	224	118	2.4
13 (So. Prudence Is.)	30	21	0.3
14 (Potter Cove, Jmstn)	39	26	1.1
15 (Newport Harbor)	2,090	183	11.3
16 (Fort Wetherill)	38	10	1.0

TABLE 2-4. Target ecological systems/species/receptors of concern.

Habitat	Ecological System/Species/Receptor of Concern
Pelagic	blue mussel (<i>Mytilus edulis</i>) mummichog (<i>Fundulus</i> spp.) winter flounder (<i>Pseudopleuronectes americanus</i>)
Epibenthic	blue mussel lobster (<i>Homarus americanus</i>)
Benthic	hard shell clam (<i>Mercenaria mercenaria</i>) soft shell clam (<i>Mya arenaria</i>) benthic community
Wetland	ribbed mussel (<i>Modiolus demissus</i>)
Avian Aquatic	osprey (<i>Pandion haliaetus</i>) herring gull (<i>Larus argentatus</i>) red-breasted merganser (<i>Mergus serrator</i>) great blue heron (<i>Ardea herodias</i>)

TABLE 2-5. Potential assessment and measurement endpoints.

Assessment Endpoint	Receptor of Concern	Measurement Endpoint
Habitat Quality	Critical habitats	Spatial distribution of habitats
Sediment Quality	Infaunal receptors	Bulk sediment toxicity to amphipods
	Epifaunal receptors	Pore water toxicity to sea urchin gametes Benthic community structure (diversity, numbers) Abundance and condition of target receptor species
Water Quality	Pelagic receptors	Growth and condition of indigenous deployed mussels
	Epifaunal receptors	Water toxicity to sea urchin gametes Abundance and condition of target receptor species
Status of Natural Resources	Resource species	Abundance and condition of target receptor species
		Abundance and condition potential prey species Bioaccumulation and trophic transfer

TABLE 2-6. Potential exposure point measurements.

Exposure Medium/ Receptor	Exposure Point Measurement
Sediment	Bulk sediment and pore water chemistry Redox potential discontinuity Geotechnical characteristics (e.g., grain size, water content) Ammonia Organic carbon SEM/AVS Pathogen abundance
Water	Water column chemistry (deployed mussel tissue residues) Dissolved oxygen Hydrographic parameters (temperature, salinity) Pathogen abundance
Biota	Tissue chemistry Pathogen abundance
Source	Seep chemistry Chemical markers Microbial markers

TABLE 3-1.**Test precision of *Ampelisca* and *Arbacia* bioassays.**

Bioassay	Toxicant	CV (%)
<i>Ampelisca</i>	Cd	40
<i>Arbacia</i>	Cu	46
	SDS	33

TABLE 3-2. Target analytes for chemical characterization.

Analyte	Sample matrix	Target method detection limits ^a
Polycyclic Aromatic Hydrocarbons (PAHs)		
	sediment biota	5 ng/g 10 ng/g
naphthalene	fluoranthene	
2-methylnaphthalene	pyrene	
1-methylnaphthalene	benz[a] anthracene	
biphenyl	chrysene	
2,6-dimethylnaphthalene	benzo [b] fluoranthene	
acenaphthylene	benzo [k] fluoranthene	
acenaphthene	benzo [e] pyrene	
1,6,7-trimethylnaphthalene	benzo [a] pyrene	
fluorene	perylene	
phenanthrene	indeno [1,2,3-cd] pyrene	
anthracene	dibenz [a,h] anthracene	
1-methylphenanthrene	benzo [ghi] perylene	
Organo-Chlorine Pesticides (OCPs)		
	sediment biota	1 ng/g 2 ng/g
Aldrin		
hexachlorobenzene		
Mirex		
o.p' - DDE		
p.p' - DDE		

TABLE 3-2. (Continued)

Analyte	Sample matrix	Target method detection limits ^a
Polychlorinated Biphenyl (PCB) Congeners		
	sediment	1 ng/g
	biota	2 ng/g
8 (2 4') ^b	126 (3 3'4 4'5)	
18 (2 2'5)	128 (2 2'3 3'4 4')	
28 (2 4 4')	138 (2 2'3 4 4'5)	
29 (2 4 5)	153 (2 2'4 4'5 5')	
44 (2 2'3 5')	154 (2 2'4 4'5 6')	
50 (2 2'4 6)	170 (2 2'3 3'4 4'5)	
52 (2 2'5 5')	180 (2 2'3 4 4'5 5')	
66 (2 3'4 4')	187 (2 2'3 4'5 5'6)	
77 (3 3'4 4')	188 (2 2'3 4'5 6 6')	
87 (2 2'3 4 5')	195 (2 2'3 3'4 4'5 6)	
101 (2 2'3 5 5')	200 (2 2' 3 3' 4 5 6 6')	
104 (2 2'4 6 6')	206 (2 2'3 3'4 4'5 5'6)	
105 (2 3 3'4 4')	209 (2 2'3 3'4 4'5 5'6 6')	
118 (2 3'4 4'5)		
Major elements		
aluminum	sediment	0.18 µg/g
	water	75.0 µg/L
	biota	0.18 µg/g
iron	sediment	0.5 µg/g
	water	20.0 µg/L
	biota	0.5 µg/g
manganese	sediment	0.01 µg/g
	water	0.50 µg/L
	biota	0.01 µg/g

TABLE 3-2. (Continued)

Analyte	Sample matrix	Target method detection limits ^a
Trace elements		
copper	sediment	0.01-0.7 µg/g
nickel	water	0.5-3.0 µg/L
chromium	biota	0.01-0.7 µg/g
lead		
silver		
cyanide		
cadmium	sediment	0.05 µg/g
	water	0.20 µg/L
	biota	0.005 µg/g
zinc	sediment	0.003 µg/g
	water	0.10 µg/L
	biota	0.003 µg/g
arsenic	sediment	0.08 µg/g
	water	3.0 µg/L
	biota	0.08 µg/g
mercury	sediment	0.125 µg/g
	water	0.10 µg/L
	biota	0.125 µg/g
Butyltins	sediment	1.0 ng Sn/g
	biota	1.0 ng Sn/g
monobutyltin		
dibutyltin		
tributyltin		

^a Sediments and tissues measured on a dry weight basis.

^b congener number (position of chlorines)

TABLE 3-3. *Ampelisca* toxicity test parameters.

Parameter	Sampling Frequency	Test Replicate Sample	Immediate Processing or Measurement
Survival	Daily	All chambers	Record number of dead amphipods and remove
Molting	Daily	All chambers	Record number of molts and remove
Temp	Daily	Water bath	Record thermometer measurement to 0.5°C
	Continuous	Water bath	Observe temperature recorder chart for variation
Salinity	Twice/test	All chambers	Record refractometer measurement to 1 ppt
Dissolved oxygen	Twice/test	All chambers	Record meter reading to 0.1 ppm
pH	Twice/test	All chambers	Record meter reading to 0.1 pH unit
Emergence	Daily	All chambers	Record number of amphipods on sediment or water surface
Tube formation	Daily	All chambers	Record unusual appearance or lack of tubes

TABLE 3-4. *Arbacia* bioassay monitoring parameters.

Parameter	Sampling frequency	Sample identity	Limits ¹
Fertilization endpoint	Termination	All reps	n/a
Salinity	Initiation	Sample	30±2 ppt
Temperature	Initiation	Sample	20±1°C

¹ Values indicate ideal conditions for performance of tests. If ambient sample parameters differ the sample will be adjusted per SOP in Mueller *et al.* (1992).

Table 4-1. Important URI Standard Operating Procedures (SOPs)

URI SOP Number	Title
2.01.001 ^a	Cleaning of Equipment for Trace Metal Analysis
2.03.005 ^a	Column Chromatography of Semivolatile Organic Analytes
2.03.007 ^a	Microwave Digestion of Organism Samples for Inorganic Analysis
2.03.008 ^a	Preparation of Water Samples for Direct Determination of Trace Metals
2.03.010 ^a	Sediment Extraction for Semivolatile Organic Analytes
2.03.011 ^a	Tissue Extraction for Semivolatile Organic Analytes
2.03.012 ^a	Total Microwave Digestion of Sediment Samples for Inorganic Analysis
2.04.002a	Gas Chromatography - Mass Spectrometry
2.04.003 ^a	Gas Chromatography, Using Electron Capture Detectors
2.04.005 ^a	Inorganic Analysis by ICP
2.04.006 ^a	Instrumental Operating Conditions for Inorganic Analysis
9011 ^b	Extraction of Sediments for Butyltin Analysis
9012 ^b	Extraction of Biological Tissues for Butyltin Analysis
9013 ^b	Quantitative Determination of Butyltins
1.01 ^c	Subtidal Sediment Chemistry Sampling
1.02 ^c	Acid Volatile Sulfide (AVS), Simultaneously Extracted Metals (SEM), and SEM/AVS
1.03 ^c	Sediment Grain Size Analysis
1.04 ^c	Low-field Susceptibility Logging
1.05 ^c	Determination of Organic Carbon Content by Loss-on-ignition
1.06 ^c	Subtidal Benthic Invertebrate Sampling, Identification, and Enumeration
1.07 ^c	Inorganic Analysis by Graphite Furnace Atomic Absorption
M1-011 ^d	Wet Digestion Procedure for the Preparation of Marine Tissue Samples for Trace Metal Analyses

a These SOPs are modified versions of the EPA Environmental Research Laboratory, Narragansett SOPs reported by Muller et al., 1992

b These SOPs are from the Geochemical and Environmental Research Group at Texas A & M University

c These SOPs are from the Inorganic Geochemistry Group at GSO/URI Quality Assurance Project Plan "Conduct of sediment toxicity tests at the Naval Education and Training Center, Newport, RI"

d. This SOP is from Microinorganics, Inc.

Table 4-2. Important SAIC Standard Operating Procedures (SOPs)

SAIC SOP Number	Title
1.01	Techniques for Extracting Pore-Water
1.02	Techniques for Diagnosis of Hematopoietic Neoplasia
2.01	Biota Sampling and Processing
3.01	Most Probable Number Method for Enumeration of <i>Clostridium Perfringens</i> in Sediment
3.02	Most Probable Number Method for Enumeration of <i>Clostridium Perfringens</i> in Sediment
3.03	Most Probable Number Method for Enumeration of Total Coliforms and Fecal Coliforms in Sediment
3.04	Most Probable Number Method for Enumeration of Total Coliforms and Fecal Coliforms in Sediment
3.05	Most Probable Number Method for Enumeration of Total Coliforms and Fecal Coliforms in Sediment
3.06	Most Probable Number Method for Enumeration of Fecal Streptococci and Enterococci in Sediment
4.01	Deployment and retrieval of caged bivalves for environmental monitoring.
4.02	Preparation of Marine Tissues for Chemical Analysis
AMP-01	<i>Ampelisca</i> collection
AMP-02	Laboratory processing of field-collected <i>Ampelisca</i>
AMP-03	Holding of <i>Ampelisca</i> prior to testing (static-renewal)
AMP-04	Press-sieving sediment for toxicity tests
AMP-05	Preparing test chambers for <i>Ampelisca</i> toxicity tests
AMP-06	Adding sediments to <i>Ampelisca</i> chambers
AMP-07	Sieving <i>Ampelisca</i> from holding jars
AMP-08	Counting <i>Ampelisca</i> into test chambers
AMP-09	Daily observations of <i>Ampelisca</i> toxicity test
AMP-10	Sieving <i>Ampelisca</i> for test breakdown
AMP-11	Picking <i>Ampelisca</i> at end-of-test
AMP-12	Picking preserved samples
SCT-01	Conducting the Sea Urchin, <i>Arbacia punctulata</i> , Fertilization Test
FLD-01	Determination of Water Column Salinity, Conductivity, Temperature, and Dissolved Oxygen Concentration using the SEABIRD CTD Profiler.
FLD-02	Collecting and Processing of Samples for Suspended Solids and Chlorophyll <i>a</i> Analyses
FLD-03	Use of the Orion Dissolved Oxygen Meter
SOD-01	Sediment and Water Oxygen Demand Measurement

TABLE 6-1. Format of site-specific addenda to the master Work Plan.

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2.2 Assessment and Measurement Endpoints of Concern, including
Contaminants and Species

2.2.1 Contaminants of Concern

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2.2.3 Assessment and Measurement Endpoints

2.3 Conceptual Model

3.0 IDENTIFICATION OF DATA NEEDS

**4.0 PLAN FOR DATA COLLECTION AND ANALYSIS (FIELD SAMPLING AND
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6.0 ECOLOGICAL EFFECTS ASSESSMENT

7.0 RISK CHARACTERIZATION

8.0 PROJECT ORGANIZATION AND RESPONSIBILITY

8.1 Project manager

8.2 Project quality assurance officer

8.3 Project principal investigators

8.4 Technical coordinator

8.5 Narragansett Bay ecorisk advisory group

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TABLE 6-2. Format of the site-specific ecological risk assessment report.

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3.2 Assessment and Measurement Endpoints, including Contaminants and Species of Concern

3.3 Site-Specific Conceptual Model

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6.4 Analysis of Toxicity Evaluations v. Observed Adverse Effects

6.5 Comparison of Exposure Point Concentrations with Criteria and Standards

6.6 Comparison of Exposure Point Concentrations with Toxicity Data

6.7 Uncertainty

Table 6-2, con't

7.0 SUMMARY AND CONCLUSIONS (optional)

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APPENDIX A

STANDARD OPERATING PROCEDURES

APPENDIX A

**Standard Operating Procedures for the
Offshore Ecological Risk Assessment at the
Naval Education and Training Center
Newport, Rhode Island**

**Graduate School of Oceanography
University of Rhode Island
Narragansett, RI 02882-1197**

and

**Science Applications International Corporation
Narragansett, RI 02882**

March 1995

POINT OF CONTACT:

John W. King
URI/Graduate School of Oceanography
South Ferry Rd.
Narragansett, RI 02882-1197

I. OBJECTIVE

All bottles, vials, pipette tips, etc. that are used for metals analyses need to be cleaned in deionized water and acid before use. This SOP describes cleaning procedures for items frequently used in the laboratory.

II. NECESSARY MATERIALS AND EQUIPMENT

- Nitric acid
- Deionized water
- Liquidnox soap

III. METHODS

A. Cleaning procedure for items used only once (pipette tips, Evergreen vials, and AA vials).

All items are rinsed in deionized water and then placed in 10% HNO₃ acid bath for 24 hours minimum. Items are then rinsed three times with deionized water and left to dry in cleanroom or cleanbench.

B. Cleaning procedure for reused items (Teflon tubes, bottles, labware)

All items are washed in Liquidnox solution and rinsed 3 times in deionized water before being placed in 1 10% nitric acid bath number 1. Items remain in the acid bath 1 for 24 hours minimum before being rinsed 3 times in deionized water and placed in a 2nd 10% nitric acid bath. After a minimum of 24 hours, items are rinsed 3 times in deionized water and left to dry in clean area.

IV. TROUBLESHOOTING

A. Nitric Acid Procedure

1. All surfaces that will be exposed to sample or reagent must be thoroughly cleaned; therefore, care must be taken to shake the containers several times to eliminate any air pockets that might be trapped inside vials.

V. STATISTICAL ANALYSIS AND DATA USAGE

Not applicable.

VI. REFERENCES

None.

GSO STANDARD OPERATING PROCEDURE 2.03.005
COLUMN CHROMATOGRAPHY OF SEMIVOLATILE
ORGANIC ANALYTES

POINT OF CONTACT:

Organic Geochemistry Laboratory
Graduate School of Oceanography
University of Rhode Island
Narragansett, RI 02882

I. OBJECTIVES

The objective of this document is to define the standard operating procedure for the preparation of silica-gel columns for the cleanup and chemical class separation of semi-volatile organic compounds in sample extracts. The resulting fractions are analyzed by gas chromatography (GC) or gas chromatography/mass spectrometry (GC/MS).

II. NECESSARY MATERIALS AND EQUIPMENT

- Pasteur pipets, (9" and 5 3/4") and Teflon® tubing connection on truncated 50 ml. pipet reservoir
- Flash evaporator (Buchler) apparatus, with heated water bath maintained at 20-30°C
- Glass pear shaped evaporation flask, 25 ml.
- Nitrogen gas, compressed, 99.99% pure
- Round bottom flasks, 100 ml. and 1 L.
- Glass vacuum flasks, 1 L.
- Glass graduated cylinders, 25 and 100 ml.
- Glass funnel short stem with Teflon® tubing attached
- Borosilicate glass vials with Teflon®-lined screw caps, 22 ml.
- Vacuum oven maintained at 130°C
- Amber bottles with Teflon®-lined screw caps, 120 ml.
- Reagents
 - Methyl alcohol, pesticide grade or equivalent
 - Methylene chloride, pesticide grade or equivalent
 - Hexane, pesticide grade or equivalent
 - Deionized Milli-Q® water, methylene chloride-extracted
 - Copper, powder purified BAKER ANALYZED 1728-01
- Glass wool, silanized
- Silica gel, Grace grade 922, 200-325 mesh size
- Buchner funnels for 11 cm filters
- Whatman (11 cm dia.) glass fiber filters (GF/C), precombusted at 450°C for 12-16 hours
- Glass pipette, 10 ml.

III. METHODS

A. Silica-Gel Preparation

1. Cleanup procedure
 - a. Add gel (vol ~ 200 ml; weight ~ 100 g) to a 1000 ml round bottom flask.
 - b. Add 500 ml of CH₂Cl₂/CH₃OH (50/50) to flask.
 - c. Reflux for one hour; turn mantle heat to low temperature (~25 on power stat) to avoid bumping, agitate occasionally, and let cool.

- d. Decant solvent and discard, add 250 ml of $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$, mix thoroughly in flask.
- e. Vacuum filter through double GF/C paper (precombusted) in Buchner Funnel, rinse with $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ (100 ml).
- f. Add 250 ml of $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ to gel in funnel without vacuum, mix with stirring rod, being careful not to tear filter; then vacuum filter.
- g. Rinse with CH_2Cl_2 (250 ml vol), pull vacuum until no drips are evident.
- h. Place in aluminum foil lined baking pan, break clumps up with stirring rod. Evaporate under vacuum for 1 hour at room temperature.
- i. Heat at 130°C for 1 hour under vacuum.
- j. Transfer to amber storage bottle labelled: Silica Gel: Pre-extracted, not activated.
- k. Activate sufficient amount (130°C for 5 hours) to last for two weeks only.

2. Activation procedure

- a. Place container under continuous vacuum for 5 hrs. at 130°C . (Date the container, store over dessicant and use within 2 weeks or reactivate.)

B. Silica-Gel Column Preparation

1. To prepare column use a 5 3/4" Pasteur pipet.
2. Put a silanized glass wool plug in tip of column. For samples containing elemental sulfur, copper powder must be used to remove the sulfur. Add copper powder (2 dipper full =2g) to the pipet and activate with 3 ml of 4N HCl (under N_2 pressure). Rinse the powder with 1ml each of MQ water, methanol, CH_2Cl_2 and hexane, in that order. Using a funnel with Teflon® tubing, pour 1.8 g of activated silica gel into pipet and tap down. Put a silanized glass wool plug on the top of the column.
3. Connect the column to the glass reservoir. Tie a Kimwipe mid-way up the column to prevent contamination of fraction vials by solvents running down outside walls of column. Clean the column into the waste jar with 30 ml CH_2Cl_2 using N_2 . Then change polarity of column by eluting 15 ml hexane, also into waste jar. (Make sure the silica gel doesn't go dry.)
4. Put a 25 ml graduated cylinder under the column, add the sample (1 to 2 ml in hexane via a 9" Pasteur pipet) from the flask through the glass reservoir and charge it to the top of the column. Let sample enter the column, then use about 1-2 ml of hexane to rinse sample from flask and reservoir onto column. When all of the hexane has entered the silica gel, add 15 ml of 98:2 hexane: CH_2Cl_2 (f_1 solvent) to the sample flask and transfer to the column reservoir via the 9" Pasteur pipet. (Do not let the 98:2 solvent stand unless it is stoppered because the CH_2Cl_2 will preferentially be lost on evaporation.) Elute (3-5 ml/min using N_2 ; 10-12 lbs/in²) into the graduated cylinder until all of the solvent enters the silica gel, making sure the gel doesn't go dry.

5. Bring the volume of the f_1 fraction up to exactly 20ml with hexane and mix well with the end of a clean 10 ml pipet. Pipet 10ml of the solution and put it into a 22ml vial labelled " f_1 & f_2 ." Cap it and put it aside for the f_2 fraction. Place the remaining solution from the graduated cylinder into a 22ml vial labelled " f_1 ." Rinse the graduated cylinder with CH_2Cl_2 and hexane (1-3ml each). Pour it into the f_1 vial. Store the f_1 vial.
6. Put another 25 ml graduated cylinder under the column and elute with 15 ml 70:30 hexane: CH_2Cl_2 (f_2 solvent) from the flask to the reservoir until all of the solvent enters the silica gel. Bring the volume of the fraction up to 20 ml with hexane and mix well with a 10ml pipet. Pipet 10ml of this solution into the vial labelled " f_1 & f_2 ." Put the remaining solution into another 22ml vial labelled " f_2 ." Rinse the graduated cylinder with CH_2Cl_2 and hexane (1-3ml each). Pour it into the f_2 vial. Store the f_2 vial.
7. Store f_1 , f_2 and f_1 & f_2 samples in the dark at room temperature until ready for GC analysis.
8. Evaporate the fractions to a small volume and add CH_2Cl_2 (to at least 50% by volume) and mix well. Add external recovery standard, then analyze by GC.

IV. TROUBLE SHOOTING

None.

V. STATISTICAL ANALYSIS AND DATA USAGE

Not applicable.

VI. REFERENCES

None.

POINT OF CONTACT:

John W. King
URI/Graduate School of Oceanography
South Ferry Rd.
Narragansett, RI 02882-1197

I. OBJECTIVE

This SOP describes a procedure for complete digestion of organism tissue analysis by atomic absorption or emission spectrophotometry. The tissue is digested in concentrated nitric acid by a two-step, microwave-assisted heating process. The first (open-vessel) step provides for substantial decomposition and dissolution of the organic tissue matrix; the second (closed-vessel) step utilizes hydrogen peroxide and the higher temperatures and pressures obtained with sealed digestion vessels to complete the digestion of the tissue in the acid, allowing measurement of the associated metals.

II. NECESSARY MATERIALS AND EQUIPMENT

- Stainless steel dissection instruments
- Deionized water
- Tissue homogenizing system (not stainless steel if chromium and/or nickel are to be analyzed)
- Advanced composite teflon digestion vessels with peel-off labels
- Laboratory scale
- Freezer
- Virtis lyophilizer
- HNO₃ (Instra-Analyzed grade), concentrated and 2M
- CEM Microwave Digestion System 2000
- H₂O₂
- Plastic tweezers
- Whatman 42 filter paper
- Filtration apparatus
- Acid-cleaned polyethylene bottle, 60 ml
- Volumetric flask, 50 ml

III. METHOD

A. Sample preparation

1. Organism samples should be thawed prior to dissection. Removed tissue specimens from shell or skin using stainless steel instruments. Rinse instruments between samples with deionized water. If required, homogenize samples using appropriate tissue homogenizing system (do not use stainless steel generators if chromium and/or nickel are to be analyzed).

2. Number the empty Teflon digestion vessels with peel-off labels and obtain the tare weight of each vessel (without the pressure relief disk).
3. Add approximately 15g of wet tissue (approximately 2.5 g dry tissue) to each vessel and reweigh, obtaining the wet gross weight. Place the vessels upright in freezer until specimens are frozen solid.
4. Freeze-dry the samples using the Virtis lyophilizer.
 - a. Drain the condenser, then pre-cool to -50C. Refrigerate sample compartment below 0C.
 - b. Place frozen specimens in sample compartment. Seal door, close vacuum release clamp and start vacuum pump. Verify that vacuum is being drawn (pressure < 1.5 torr).
 - c. Freeze-dry specimens for 48 hr. at -40C, then turn off shelf heat and hold for 24 hr. at 45C.
5. Remove the vessels from the freeze dryer and weigh again, obtaining the dry gross weights for the samples.

B. Microwave digestion

1. Open-vessel digestion

- a. Add 15 ml of concentrated HNO₃ (Instra-Analyzed grade) to each sample vessel and close cap, without pressure relief disks, hand-tight. If bubbling or foaming occurs, allow samples to sit at room temperature until foaming subsides (1 hr).
- b. Load vessels into carousel, place carousel into microwave oven and close door. Begin carousel rotation, making sure oven exhaust fan is operating.
- c. Program MDS-81:

	Time(min)	6 vessels	Power level 8 vessels	12 vessels
S-1:	3:00	25%	30%	35%
S-2:	5:00	35%	40%	55%
S-3:	5:00	50%	60%	75%

and press START to initiate microwave digestion.

- d. After program has completed run, remove sample carousel from MDS-81 and place in hood to cool.

2. Closed-vessel digestion

- a. Remove cap from each vessel and add 3 ml H₂O₂ to vessel. Place pressure relief disk, ring side up, on top of lower portion of vessel and replace cap and tighten.
- b. Place vessels in carousel. Insert vent tube into each vessel neck and tighten nut. Insert free end of tube into vent trap in center of carousel and return carousel to oven. Insure that venting fan is operatin and begin carousel rotation.
- c. Program CEM 2000:

	Time(min)	Power level		
		6 vessels	8 vessels	12 vessels
S-1:	6:00	40%	50%	60%
S-2:	2:00	0%	0%	0%
S-3:	5:00	50%	60%	80%

and press START to initiate microwave digestion.

d. After program is completed, remove carousel from CEM2000 and place in hood to cool (minimum 1 hour). When vessels are cool to touch, remove vent tubes and CAREFULLY vent vessels manually to release pressure. If venting is too vigorous, allow to cool longer and vent again. Repeat until no more venting occurs.

e. Remove caps from vessels. Invert cap and pressure relief disk over vessel and rinse with deionized water, allowing rinse to drain into vessel. Add 15 ml of deionized water to vessel.

C. Sample filtration and dilution

1. Using plastic tweezers, place circle of Whatman 42 filter paper into filtration apparatus. Wash filter with 2 M HNO₃. Place 60-ml acid-cleaned, polyethylene bottle and vacuum gasket under filtration apparatus and apply vacuum. Filter digested sample solution through filter paper into bottle. Rinse the digestion vessel with deionized water and pour through filter as well. Repeat rinse/filtration. Holding sample bottle, release vacuum and remove bottle.

2. Pour combined filtrates from bottle into 50ml volumetric flask. Rinse bottle and use the rinse to dilute solution in flask to the volume mark. Discard any remaining rinse solution in bottle. Return the sample solution to the bottle and label bottle appropriately.

IV. TROUBLE SHOOTING

Discussed in methods.

V. STATISTICAL ANALYSIS AND DATA USAGE

Not applicable.

VI. REFERENCES

None.

IV. TROUBLESHOOTING

Discussed in methods.

V. STATISTICAL ANALYSIS AND DATA USAGE

Not applicable.

VI. REFERENCES

None.

GSO STANDARD OPERATING PROCEDURE 2.03.010
SEDIMENT EXTRACTION FOR SEMIVOLATILE ORGANIC
ANALYTES

POINT OF CONTACT:

Organic Geochemistry Laboratory
Graduate School of Oceanography
University of Rhode Island
Narragansett, RI 02882

I. OBJECTIVES

The objective of this document is to define the standard operating procedure for the extraction of semi-volatile organic compounds from sediment samples. The extracts are further cleaned up by silica gel chromatography procedures prior to analysis by gas chromatography (GC) or gas chromatography/mass spectrometry (GC/MS).

II. NECESSARY MATERIALS AND EQUIPMENT

- Stainless steel or Teflon®-coated spatula
- Aluminum weighing pans, 57 mm diam.
- Mettler Analytical Balance capable of weighing to 0.1 mg
- Drying oven maintained at 105-120°C
- Glass round bottom flasks with ground glass joints, 250- and 500-ml
- Cork supports for glass round bottom flasks
- Glass funnel
- Top-loading balance capable of weighing to 0.01 g
- Microliter syringes or micropipets, solvent rinsed
- Hemispherical heating mantles and transformer
- Glass reflux condensers with ground glass joints
- Buchner funnels for 11 cm filters
- Glass vacuum flasks, 500 and 1000 ml.
- Whatman GF/C glass fiber filters (11 cm diam.) precombusted at 450° C for 12-16 hours
- Glass separatory funnels, 500 and 1000 ml.
- Glass graduated cylinders, 100- and 500-ml
- Buchler flash evaporator apparatus, with heated water bath maintained at 20-30° C
- Glass desiccator and desiccant
- Top-loading balance capable of weighing to 0.01 g
- Reagents
 - Internal Standards in acetonitrile, to be added to each sample prior to extraction.
 - Acetonitrile, pesticide grade or equivalent
 - Deionized Milli-Q® water, methylene chloride-extracted
 - Hexane, pesticide grade or equivalent

III. METHODS

1. Homogenize the thawed sediment sample with a stainless steel or Teflon®-coated spatula. With an analytical balance, weigh approximately 1 to 5 grams of moist sample into two tared aluminum pans for duplicate dry/wet determination. Dry for at least two hours at 105-115°C. Cool in a desiccator and dry to constant weight. The difference in the percent dry of the duplicate samples should be less than 5%.

2. Using a top loading balance weigh approximately 10 to 20 g of the homogenized sample into a solvent rinsed glass round bottom flask. A glass funnel may be necessary depending on the viscosity of the sample. The amount of sample may be adjusted based on expected contaminant concentrations or detection limits required. Record exact unit weight of the sample.
3. Add Surrogate Internal Standards (SIS) as required: A mixture of DBOFB, CB198, and OCN in acetonitrile for PCBs and OCPs and a mixture of d8-naphthalene, d-10 biphenyl, d10-acenaphthene, d10-anthracene, and d12-perylene in acetonitrile for PAHs. The amount of SIS added is dependent on the expected contaminant concentrations and should be equivalent to those concentrations.
4. Add enough acetonitrile to cover the sediment (100 to 200 ml.).
5. Reflux the sample for 2 hours. Swirl the flask two or three times during reflux.
6. Cool sample (store in darkness if necessary).
7. Vacuum filter onto a Buchner funnel containing an 11cm precombusted Whatman GF/C glass fiber filter into a vacuum flask. Rinse the round bottom flask and the filter paper with about 100ml of acetonitrile.
8. Transfer the acetonitrile filtrate to a glass separatory funnel. Add an amount of methylene chloride extracted deionized water at least equal to the volume of acetonitrile filtrate.
9. Add a volume of hexane that is at least 10% of the water-acetonitrile mixture's volume. Shake and vent for two minutes, let stand until the two phases separate.
10. Decant the hexane phase from the top layer of the separatory funnel into a solvent rinsed glass round bottom flask. Avoid decanting any of the water/acetonitrile phase and if necessary leave a small amount of the hexane phase in the separatory funnel.
11. Repeat steps 9 and 10 two more times combining the hexane phases in one glass round bottom flask.
12. Store the combined hexane phase in darkness if necessary.
13. Roto-evaporate the hexane phase down to 1-2 ml and fractionate the sample following the Column Chromatography SOP 2.03.005.

IV. TROUBLESHOOTING

None.

V. STATISTICAL ANALYSIS AND DATA USAGE

Not applicable.

VI. REFERENCES

None.

GSO STANDARD OPERATING PROCEDURE 2.03.011
TISSUE EXTRACTION FOR SEMIVOLATILE ORGANIC
ANALYTES

POINT OF CONTACT:

Organic Geochemistry Laboratory
Graduate School of Oceanography
University of Rhode Island
Narragansett, RI 02882

I. OBJECTIVES

The objective of this document is to define the standard operating procedure for the extraction of semi-volatile organic compounds from tissue samples. The extracts are further cleaned up by silica gel chromatography procedures prior to analysis by gas chromatography (GC) or gas chromatography/mass spectrometry (GC/MS).

II. NECESSARY MATERIALS AND EQUIPMENT

- Waring Blender motor
- Stainless steel blender vessel
- Top-loading balance capable of weighing to 0.01 g.
- Mettler analytical balance capable of weighing to 0.1 mg.
- Aluminum weigh dishes, 57 mm. diameter
- Stainless steel or Teflon®-coated spatula
- Glass round-bottom flasks, 250 ml.
- Drying oven maintained at 105-120°C.
- Flash evaporator (Buchler) apparatus, with heated water bath maintained at 20-30°C.
- Nitrogen gas, compressed, 99.99% pure
- Glass funnels, 122 mm. diameter, stem length 111 mm. and OD 15 mm.
- Glass graduated cylinders, 100 ml.
- Glass separatory funnels, 500 ml.
- Glass vacuum flasks, 500 ml.
- Microliter syringes or micropipets, solvent rinsed
- Whatman (11 cm dia.) glass fiber filters(GF/C), precombusted at 450°C. for 12-16 hours
- Heating mantles and transformer
- Buchner funnel for 11 cm. filters
- Glass volumetric flasks, 100 ml.
- Amber bottles with Teflon®-lined screw caps, 120 ml.
- Glass desiccator and desiccant
- Reagents
 - Acetonitrile, pesticide grade or equivalent
 - Hexane, pesticide grade or equivalent
 - Deionized Milli-Q®, methylene chloride-extracted
 - Internal Standards in acetonitrile, to be added to each sample prior to extraction.

III. METHODS

1. Transfer thawed tissue to the previously cleaned, solvent rinsed and tared Waring Blender vessel so that approximately 35-45 grams of tissue sample is present.
2. Blend tissue sample until homogeneous, e.g. 2-3 pulses of 10-15 seconds each. Add small amounts of deionized water, if necessary, to improve blending and recovery of tissue, especially if the amount of sample available is less than 30 grams.
3. With the analytical balance, weigh approximately 1 to 5 grams of blended tissue into two preweighed aluminum dishes for replicate % moisture determinations. Dry for at least two hours at 105-120°C., cool in a desiccator, and weigh. Repeat as necessary to dry sample to constant weight. The difference in the percent dry of the duplicate samples should be less than 5%.
4. Transfer the rest of the blended sample (30-40 g) with a spatula into a tared organically cleaned, round-bottom flask by means of a long stemmed glass funnel that drops the wet sample into the flask center. Weigh this flask with sample on the top loading balance. Record exact wet weight of all samples.
5. Add Surrogate Internal Standards (SIS) as required: A mixture of DBOFB, CB198, and OCN in acetonitrile for PCBs and OCPs and a mixture of d8-naphthalene, d-10 biphenyl, d10-acenaphthene, d10-anthracene, and d12-perylene in acetonitrile for PAHs. The amount of SIS added is dependent on the expected contaminant concentrations and should be equivalent to those concentrations.
6. Add enough acetonitrile to cover the sample (100-200 ml.).
7. Reflux the sample for 2 hours. Swirl the flask two or three times during reflux.
8. Cool sample (store in darkness if necessary).
9. Vacuum filter onto a Buchner funnel containing an 11 cm. precombusted Whatman GF/C glass fiber filter into a vacuum flask. Rinse the round bottom flask and the filter paper with an additional 50-100 ml. of acetonitrile.
10. Transfer the acetonitrile filtrate into a clean separatory funnel. Add an amount of methylene chloride extracted deionized water at least equal to the volume of acetonitrile filtrate.
11. Partition the extract with 50-60 ml. hexane; shake and vent, let stand until phases separate. Decant the top layer of hexane into a round-bottom flask. Avoid decanting any of the acetonitrile/water mix (bottom layer). Repeat partition step twice more combining the hexane phases in the round-bottom flask. Store in darkness if necessary.

12. Roto-evaporate hexane to less than 100 ml.; bring to volume with hexane in a 100 ml. volumetric flask; store the 100 ml. sample solution in a labeled brown bottle in the dark at room temperature. Flush briefly with nitrogen and seal tightly.
13. Remove exact aliquots as needed for column chromatography or total lipid analysis, flush very briefly with nitrogen and seal tightly after each opening.
14. Roto-evaporate the aliquot for lipid analysis to a small volume and transfer to a pre-weighed aluminum weighing pan. Allow to dry at room temperature for several hours, store in a desiccator and dry to constant weight on the analytical balance.
15. Roto-evaporate the aliquot for column chromatography down to 1-2 ml and fractionate the sample using the column chromatography SOP 2.03.005.

IV. TROUBLE SHOOTING

None.

V. STATISTICAL ANALYSIS AND DATA USAGE

Not applicable.

VI. REFERENCES

None.

POINT OF CONTACT:

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I. OBJECTIVE

This SOP describes the procedure for complete digestion of sediments for determination of metals concentrations. Because the digestion is complete, the concentrations measured are the total metal concentrations in the sediment, including both anthropogenic and natural background components.

II. NECESSARY MATERIALS AND EQUIPMENT

- Concentrated nitric acid
- Hydrofluoric acid
- Boric acid
- CEM Advanced Composite Teflon Digestion vessels
- Freezer
- Virtis freeze drier
- Laboratory scale
- Protective clothing
 - Labcoat
 - Polyethylene apron
 - Neoprene gloves
 - Safety goggles (not glasses)
 - Face shield
- CEM Microwave Digestion System Model 2000
- Fume hood
- 50 ml volumetric flask
- Deionized water
- Clean, acid-stripped polyethylene bottle

III. METHOD

Complete digestion of the mineral matrix is accomplished by the use of concentrated nitric and hydrofluoric acid, which must be neutralized by reaction with boric acid prior to analysis in order to prevent etching of glassware in the analytical instrumentation.

A. Sample preparation

1. Sediments should be thawed and homogenized using appropriate equipment prior to subsampling for analysis.
2. Number the empty Teflon digestion vessels with peel-off labels and obtain the tare weight of each vessel (without the pressure relief disk).
3. Add approximately 1.0 - 1.5 g of wet sediment to each vessel and reweigh, obtaining the gross weight. Place the vessels upright in freezer until the sediments are frozen solid.
4. Freeze-dry the samples using the Virtis freeze drier.
 - a. Drain the condenser, then pre-cool to -50C. Refrigerate sample compartment below 0 C.
 - b. Place frozen specimens in sample compartment. Seal door, close vacuum release clamp and start vacuum pump. Verify that vacuum is being drawn (pressure <1.5 torr).
 - c. Freeze-dry specimens for 48 hr. at - 40 C, then turn on shelf heat and hold for 24 hr at 45C.
5. Remove the vessels from the freeze drier and weigh again, obtaining the dry gross weights.

B. Microwave digestion

1. Before digesting the sediment samples, the chemist must be wearing appropriate protective clothing: labcoat, polyethylene apron, neoprene gloves, safety goggles (not glasses) and face shield.
2. Add 3.0 ml of concentrated nitric acid (HNO₃) to each vessel. Swirl slightly to wet sediment and check for reaction with sediment, e.g. foaming or bubbling. When no reaction is evident, add 3.0 ml of concentrated hydrofluoric acid (HF) to each sample vessel. Place pressure relief disk, ring side up, on top of lower portion of vessel and replace and tighten cap.
3. Place vessels in carousel. Insert vent tube into each vessel neck and tighten nut. Insert free end of tube into vent trap in center of carousel and return carousel to oven. Insure that venting fan is operating and begin carousel rotation.

4. Program CEM Microwave Digestion System 2000

# of vessels =	4 vessels	6 vessels	8 vessels
Step	Time		Power %
S-1:	3:00	40	55
S-2:	6:00	75	100
S-3:	20:00	45	60

(* digest for 7:30)

and press START to initiate microwave digestion.

5. After program is completed, remove carousel from CEM 2000 and place in hood to cool (minimum 30 minutes). When vessels are cool to touch, remove vent tubes and CAREFULLY vent vessels manually to release pressure. If venting is too vigorous, allow to cool longer and vent again. Repeat until no more venting occurs.

6. Remove caps from vessels. Ad 30 ml of 5% boric acid solution to each vessel, replace pressure relief disk and cap, and tighten cap, as above.

7. Program CEM-2000:

# of vessels =	4 vessels	6 vessels	8 vessels
Step	Time		Power %
S-1:	15:00	40	60

and press START.

8. After program is completed, remove carousel from CEM2000 and place in hood to cool. When vessels are cool, remove vent tubes and vent vessels. Observe same precautions as above.

C. Sample filtration and dilution

1. Transfer contents of each digestion vessel without filtering into a 50 - ml volumetric flask.
2. Rinse the vessel with deionized water, adding the rinse to the volumetric flask. Dilute with deionized water to the volumetric flask.
3. Pour the sample solution into a clean, acid-stripped polyethylene bottle and label the bottle appropriately.

IV. TROUBLE SHOOTING

Discussed in methods section.

V. STATISTICAL ANALYSIS AND DATA USAGE

Not applicable.

VI. REFERENCES

None.

GSO STANDARD OPERATING PROCEDURE 2.04.002
GAS CHROMATOGRAPHY-MASS SELECTIVE DETECTOR

POINT OF CONTACT:

Organic Geochemistry Laboratory
Graduate School of Oceanography
University of Rhode Island
Narragansett, RI 02882

I. OBJECTIVE

The objective of this document is to define and outline the standard operating procedure for analyzing sample extracts for polycyclic aromatic hydrocarbons (PAHs) using high resolution gas chromatography with mass selective detection in the electron impact / selected ion monitoring mode.

II. NECESSARY MATERIALS AND EQUIPMENT

- Hewlett Packard 5890 Series II Gas Chromatograph with a splitless injection port.
- Hewlett Packard 5971A Mass Selective Detector.
- Hewlett Packard Data Analysis System (Vectra QS/20) with HP G1034C MS ChemStation Software.
- Hewlett Packard 59822B Ionization Gauge Controller
- Alcatel 2005 High Vacuum Pump
- J&W Scientific DB-5MS 30-meter narrow bore fused silica capillary column (0.25 mm I.D. and 0.25 micron film thickness).
- 99.999% Helium carrier gas.
- Hamilton gas tight 10 microliter syringe.
- Research grade quantitative(NIST) and internal standards.

III. OPERATION

A. Instrument checks made prior to data collection

1. Gas supply

- a. Check gas cylinder pressures. Replace tank if pressure is less than 100 psi.
- b. Check head pressure gauge on front panel of instrument. Gauge should read 55 kPa (8.0 psi) at 85°C; adjust to correct setting if reading is high; check for leaks if pressure is low.
- c. Replace injection port septum at least twice a week (Monday and Thursday).
- d. Check septum purge and split flows. Adjust to ~2 and 10.7 ml/min, respectively, as necessary.

2. Vacuum

- a. Check the ion gauge, the vacuum should be less than $3.0E-5$ Torr. If a leak is suspected, check the septum nut, column fittings, and the MSD interface for leaks, tighten as necessary.
- b. If the leak persists, refer to the HP Mass Selective Detector manual for guidance.

3. Mass Selective Detector

- a. Run a standard usertune. Refer to the HP Mass Selective Detector manual for interpretation of the tune results. If the tune passes the criteria, save this tune under the filename UTUNE. If the tune fails, refer to the HP Mass Selective Detector manual for guidance.

B. Data Collection

- a. Load the appropriate method, SIMEXTND, in the top level of the Chemstation software.
- b. Pull down the run method window. Fill in the file number (derived from the date, i.e., the first run of August 1, 1994, is 010894A.D), operator's initials, sample name, and the injection volume. Record this information and also the ion gauge reading in the GC-MS logbook. Click on OK and proceed to the sample injection.
- c. The following method information is downloaded to the GC. The not ready light on the GC will continue to flash until all of the initial conditions are reached:

Initial Oven Temperature	85°C
Solvent Delay	7.00 minutes
Initial Hold Time	7.03 minutes
Primary Temperature Ramp	20°C/minute to 195°C
Primary Hold Time	0.5 minute
Secondary Temperature Ramp	16°C/minute to 260°C
Secondary Hold Time	4.0 minutes
Tertiary Temperature Ramp	11°C/minute to 325°C
Tertiary Hold Time	13 minutes
Total Run Time	40.00 minutes
Injector port Temperature	325°C
Detector Temperature(Interface)	320°C
Column Head Pressure	55 kPa at 85°C
Purge Activation Time	1.50 minutes

- d. Once the not ready light has stopped flashing, the sample can be injected and the START button on the GC is pressed. Click NO on the OVERRIDE SOLVENT DELAY window.

C. Data Analysis

- a. Following the run, the analytes are identified and a report is generated by the data system. Proper peak identification and integration can be verified and manually modified if necessary, as allowed by the software. Any manual changes must be saved and a new report must be regenerated.
- b. A customized Excel spreadsheet, PAH&UCM, is loaded under the LAYOUT CUSTOM REPORT window. Sample concentrations (ng/g) are calculated once the sample weight and amount of internal standard are entered onto the spreadsheet. A copy of the spreadsheet is printed and stapled to a printout of the sample's chromatogram.

IV. QUALITY ASSURANCE AND TROUBLESHOOTING

1. Chromatograms of standards are compared to posted references. Peak identifications, resolution and shapes are inspected. Calculated standard amounts are checked for accuracy. Other abnormalities, such as spurious or extra peaks, and rising or falling baselines are examined. Response factors are compared to previous runs. Blanks are checked for the presence of interferences or analytes of interest. Unknown samples are compared to standards to check peak identifications.
2. Refer to the GSO GC Troubleshooting notebook and the manufacturers' manuals for guidance in troubleshooting.

V. STATISTICAL ANALYSIS AND DATA USAGE

Not applicable.

VI. REFERENCES

None.

POINT OF CONTACT:

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I. OBJECTIVES

The objective of this document is to define and outline the standard operating procedure for analyzing sample extracts for polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) utilizing high resolution gas chromatography with electron capture detection.

II. NECESSARY MATERIALS AND EQUIPMENT

- Hewlett Packard 5890 Gas Chromatograph with a splitless injection port and a linearized ^{63}Ni electron capture detector.
- 60 meter J&W Scientific DB-5 narrow bore fused silica capillary column (0.25 μ film thickness, 0.25 mm i.d.).
- Hewlett Packard Data Analysis System (HP3365 Series II Chemstation DOS Series software, Vectra VL2 4/50 hardware)
- 99.999% Helium carrier gas and Argon/Methane (95/5%) detector gas.
- Septum Thermogreen™ LB-2, 9.5 mm. (Supelco, Inc.).
- Hamilton gas tight 5.0 microliter syringe.
- Research grade quantitative (NIST) and internal standards.

III. OPERATION

A. Instrument checks made prior to data collection

1. Gas supply

- a. Check gas cylinder pressures. Replace tank if pressure is less than 100 psig.
- b. Check head pressure gauge on front panel of instrument. Gauge should read 175 kPa (25.4 psi) at 100°C; adjust to correct setting if reading is high; check for leaks if pressure is low. This setting provides for a carrier gas flow of approximately 1.5 ml/min.
- c. Replace injection port septum at least twice a week (Monday and Thursday). Check septum nut and column fittings for leaks with leak detector and tighten as necessary.
- d. Check the auxiliary gas flow. A flow of at least 35 ml/min is required. Argon/Methane flow should be approximately 5.5 ml/min.
- e. Check septum purge and split flows. Adjust to ~0.4 and 40 ml/min, respectively, as necessary.

2. Instrument output signal
 - a. Display the analog output signal from the detector on the LED panel of the GC.
 - b. Record the value in the instrument log book, and check for consistency with previous readings.
3. Instrument operating parameters
 - a. Temperature programs and run times are stored as method files. The following conditions are required for the analysis of PCBs and OCPs:

Injection Port Temperature	315°C
Detector Temperature	325°C
Initial Oven Temperature	100°C
Initial Hold Time	1 minute
Primary Temperature Ramp	11°C/minute to 130°C
Primary Hold Time	2 minutes
Secondary Temperature Ramp	1.5°C/minute to 190°C
Secondary Hold Time	6 minutes
Tertiary Temperature Ramp	2.3°C/minute to 325°C
Final hold time	10 minutes
Total Run Time	120.4 minutes
Purge Activation Time	0.70 minute

- b. Load the appropriate method, PCB.MTH, into the GC/ECD computer/data system.
 - c. Load the appropriate sequence, pcb.seq, into the computer. Continue stepwise loading the appropriate information into the following data system windows: under Sequence-Edit Sequence Parameters, Edit Sequence Table, Edit Sample Table, and Edit Sample Log Table; under Data Analysis- Edit Calibration Settings and Specify Report; and under Run Control- Sample Info.
 - d. Recheck the last window under Sequence- Edit Sample Log Table; if okay than go under Method- Save as PCB.MTH (overwrite). Then under Run Control- Run Sequence (OK).
- B. Data system setup
1. Scheduling of standards and samples
 - a. Setting up the instrument is accomplished by following the step-by-step OGL instructions written for the GC/ECD INJECTIONS.
 - b. The Standardization of the two Methods- PCB.MTH and PEST.MTH, used are each based on three calibration points. Every week one calibration of each method is injected and the result is compared with the calibration curve.
 - c. Manual injections (1-2 µl) are made using a dedicated syringe (5.0µl) that has been rinsed repeatedly with methanol, methylene chloride and hexane (2X, 2X and 4X).

C. Instrument startup and data collection

1. Visually recheck tank regulator gauges and instrument settings to ensure proper settings. When satisfied all information in the data system is accurate, Run Sequence under Run Control.
2. When light on GC control panel goes out, inject sample and press 'Start' on the GC control panel to start the data collection; record the injection information in the GC/ECD Log Book.

D. Peak identification and quantitation

1. Peak identification is accomplished by automated routines. Identifications are based on comparison of retention times of actual standards to unknown peaks. Multilevel standards are calibrated to generate a linear regression curve of response according to the manufacturer's instructions. After a calibration curve has been generated, the samples are analyzed. Analytes are quantitated based on the peak areas for the analytes and internal standard, the amount of the internal standard, and the response factors generated from the calibration curve. Chromatograms and data reports are generated for each sample and standard.

IV. QUALITY ASSURANCE AND TROUBLESHOOTING

1. Chromatograms of standards are compared to posted references. Peak identifications, resolution and shapes are inspected. Calculated standard amounts are checked for accuracy. Other abnormalities, such as spurious or extra peaks, rising or falling baselines, and negative spiking are examined. Response factors are compared to previous runs. Blanks are checked for the presence of interferences or analytes of interest. Unknown samples are compared to standards to check peak identifications.
2. Refer to the GSO GC Troubleshooting notebook and the manufacturer's manuals for guidance in troubleshooting.

V. STATISTICAL ANALYSIS AND DATA USAGE

Not Applicable.

VI. REFERENCES

None.

POINT OF CONTACT:

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I. OBJECTIVE

This procedure describes the analysis of metals by inductively coupled plasma (ICP) atomic emission spectrometry.

II. NECESSARY MATERIALS AND EQUIPMENT

- ARL model 3410 ICP spectrometer.

III. METHOD

A. Instrumental startup

1. Verify proper operating conditions:

- a. Torch gas supply valve, pressure = 75 psi
- b. Purge gas flow = 3 SCFH
- c. Spray chamber drain bucket < 75% full
- d. Reservoir for autosampler rinse well full

2. Start sample pump

- a. Inspect tubing for flexibility, absence of cracks or excessive discoloration.
- b. Stretch tubing around pump head and close clamp.
- c. Place autosampler tip in deionized water and turn on pump.
- d. Verify steady, continuous uptake of water.

3. Ignite plasma

- a. Press "START Torch" button on front of ICP.
- b. Check gas flow controls inside torch box:

1. Coolant = 23 psi
2. Plasma = 22 psi
3. Carrier = 50 psi

- c. Record time TORCH ON in instrument usage log & allow plasma to stabilize > 15 minutes (this is a good time to edit autosampler files, if needed).

1. If plasma ignites, but is extinguished when carrier gas flow initiates, allow gas flows to purge torch and repeat (which step); plasma should stay lit by 2nd or 3rd try.
2. If plasma fails to light or stay lit:
 - a. Verify application of RF power.
 - b. Clean & gap igniter and repeat.
 - c. If plasma still won't light, service call may be required.

4. Power up EPIC (ICP instrument control software) on computer.
 - a. Turn on power to computer, printer and display, if needed.
 - b. Turn up intensity and/or contrast on display as needed.
 - c. Select "ICP Operations" from menu (F1).

- B. Calibrate wavelength driver.
 1. Select "Zero Calibration" from menu (F2).
 2. Perform zero order calibration (F7).
 3. While zero order calibration is performed, record RF generator parameters in instrument usage log.
 4. Record reference wavelength position in instrument usage log.
 5. Return to ICP operations menu (F10).

- C. Normalize the appropriate task.
 1. Select "Normalization" from menu (F7).
 2. Select task, if necessary (F1).
 3. Enter number of replicates (F3) (usually 3).
 4. Select hard copy logging if printout desired (F6).
 5. Perform normalization (F8).
 - a. If using autosampler:
 1. Load wavelength calibration solution and normalization standards into autosampler.
 2. Advance autosampler to place first solution in position for autosampler.
 3. Answer prompts:
 - a. Run uninterrupted? Y
 - b. Special wavelength or Calibration? S
 - c. Special Cal. sln queued up? Y
 - b. if not using autosampler:
 1. Calibrate wavelengths.
 - a. Special Wavelength or Calibration? S
 - b. Place autosampler tip in wavelength calibration solution
 - c. Special Cal. sln queued up? Y

2. Run normalization stds.

- a. Select normalization standard to run and enter standard number at prompt.
- b. Place autosampler tip in standard solution and respond "Y" to prompt.

c. Calculate new normalization coefficients.

1. After all normalization standards have been run, enter "-1" for standard number.
2. Current and new coefficients are displayed; coefficients deviating more than + 10% from original calibration highlighted in red.
3. Continue (F10).
 - a. If all or most of coefficients are within 10%, accept new coefficients "Y".
 - b. If many coefficients deviate by more than 10% or are erratic, do not accept the new coefficients "N"; recalibration or repeat of normalization necessary.

6. Return to ICP operations (F10)

D. Analyze samples.

1. Select task, if necessary (F2).
2. Select autosampler file to be used, if any (F3).
3. Enter the number of replicates (F4) (usually 3).
4. Select results file to store analytical results (F5).
 - a. Select results file to append results to existing file (F2).
 - b. Enter file name to create new file (F5).
 - c. Return to analysis menu (F10).
5. Select hard copy logging to get printout during analysis.
6. Proceed to analysis options menu (F8) and select options as applicable, e.g. display intensities, dilution correction, etc.
7. Proceed to sample analysis menu (F8).
8. Begin analysis of sample(s).
 - a. if not using autosampler:
 1. Enter sample ID (F1) and weight and dilution corrections (F2 & F3), if desired.
 2. Place autosampler tip in sample solution and initiate analysis (F8).
 3. Repeat (which step) and (which step) for each sample.
 - b. If using autosampler:
 1. Advance autosampler to place first solution in position for autosampler.
 2. Initiate analysis of samples (F8).

3. After autosampler file has run through, more samples may be analyzed manually (I.D.8.a or with another autosampler file.

d. Return to ICP operations (F10) and EPIC (F10)

E. Shutdown

1. Pump deionized H₂O through pump and spray chamber for > 15 minutes.
2. Stop pump, release clamp and remove tubing from pump head.
3. Press "STOP Torch" button and record time TORCH OFF in instrument usage log.
4. Turn display intensity all the way down.

IV. TROUBLE SHOOTING

Discussed in methods.

V. STATISTICAL ANALYSIS AND DATA USAGE

Not applicable.

VI. REFERENCES

None.

POINT OF CONTACT:

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I. OBJECTIVE

The conditions given below describe the particular instrumental parameters derived for atomic absorption and emission analysis of environmental samples at ERL-N.

II. NECESSARY MATERIALS AND EQUIPMENT

- ARL Model 3410 ICP spectrometer
- Perkin-Elmer 4100ZL graphite furnace atomic absorption spectrophotometer

III. METHODS

Where conditions for a particular element and instrument are not specifically provided, the instrument manufacturer's recommended operating conditions and parameters are used.

A. Inductively Coupled Plasma Operating Conditions

Element	Analytical Wavelength (nm)	Bkgd. Correction Wavelength(s) (nm)	Detection Limit (ug/ml)
Cu	324.754	324.793	0.010
Zn	213.856	213.820	0.005
Cr	205.552	205.588	0.010
Pb	220.353	220.311, 220.401	0.100
Ni	231.604	231.568	0.020
Cd	228.880	228.902	0.003
Mn	257.610	257.646	0.005
Fe	273.955	274.000	0.020 (sediments)
	259.940	259.985	0.020 (organisms)

RF Power: 650 W forward, < 8 W reflected
Argon gas flows: Coolant 6.5 L/min
Plasma 1.0 "
Nebulizer 0.7 "

Sample solution pumping rate: 1.75 ml/min

B. Graphite Furnace Atomic Absorption Operating Conditions

Element	Wavelength (nm)	Slit (nm)	Ash temp deg C	Atom temp deg C	Matrix Modifier
Al	309.3	0.7	1200	2300	15µg Mg(NO ₃) ₂
Ag	328.1	0.7	800	1500	5µg NH ₄ H ₂ PO ₄
As	193.7	0.7	1200	2000	5µg Pd + 3µg Mg(NO ₃) ₂
Cd	228.8	0.7	700	1400	50µg PO ₄ + 3µg Mg(NO ₃) ₂
Cu	324.8	0.7	1200	1900	5µg PO ₄ + 3µg Mg(NO ₃) ₂
Cr	357.9	0.7	1500	2300	15µg Mg(NO ₃) ₂
Fe	248.3	0.2	1400	2100	15µg Mg(NO ₃) ₂
Mn	279.5	0.2	1300	1900	5µg PO ₄ + 3µg Mg(NO ₃) ₂
Ni	232.0	0.7	1100	2300	
Pb	283.3	0.7	850	1500	50µg PO ₄ + 3µg Mg(NO ₃) ₂
Zn	213.9	0.7	700	1800	5µg Mg(NO ₃) ₂

Analyses are performed using THGA Graphite tube with integrated L'vov-type platform, maximum power heating, zero-gas flow during atomization and Zeeman background correction. Peak areas used for calibration and quantitation. All analyses utilize 20 ul sample injections + matrix modifier dependent on the element.

IV. TROUBLESHOOTING

Discussed in methods section.

V. STATISTICAL ANALYSIS AND DATA USAGE

Not applicable.

VI. REFERENCES

None.

Geochemical and Environmental Research Group
STANDARD OPERATING PROCEDURES

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SOP-9011

EXTRACTION OF SEDIMENTS FOR BUTYLTIN ANALYSIS

Written by

Terry L. Wade

This document presents the protocol, materials, and quality control used in the performance of the above analysis.

Laboratory Manager
Terry L. Wade

Date

Quality Control Officer
Thomas J. Jackson

Date

Rev. 2

Approved

Mellan C. Jenne 5/27/92

May 27, 1992

EXTRACTION OF SEDIMENTS FOR BUTYLTIN ANALYSIS .

1.0 INTRODUCTION

Assessment of the environmental impact of butyltins requires their measurement in sediments at trace levels (parts per billion to parts per trillion).

This standard operating procedure provides a precise and accurate method to quantitatively determine butyltin compounds in sediments. Freeze-dried samples are serially extracted with 0.2% tropolone in methylene chloride. The extract is then hexylated with a Grignard reagent. The hexylated extract is dried and concentrated. A silica/alumina column cleanup step is used before the instrumental analysis to remove matrix interferences. The protocol is designed for 20 gram samples, but sediment samples of other sizes may be collected and extracted by appropriately adjusting the volume of tropolone/methylene chloride used for the extraction. The extract is then submitted for analysis of butyltins by GERG SOP-9013.

2.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

2.1 Sample Collection

Collect and place sediment samples in precleaned mason jars.

2.2 Sample Storage

Store sediment samples in the dark at or below -20°C. Store sample extracts in the dark at 4°C.

3.0 INTERFERENCES

Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to false positives in GC/FPD detection. All materials used in this method are routinely demonstrated to be free from interferences by processing procedural blanks identical to samples (one blank per 12 samples or each batch whichever is more frequent).

Matrix interferences result from the co-extraction of compounds other than the analytes of interest. Previous analyses of sediments indicate that matrix interferences are generally low.

4.0 APPARATUS AND MATERIALS

4.1 Labware and Apparatus

Clean glassware by detergent (micro cleaning solution) washing with water and rinsing with tap water. Then combust the glassware in a muffle furnace at 400°C for at least 4 hours. Solvent rinses of methanol to dry followed by methylene chloride may be substituted for the muffle furnace heating when determined to be appropriate by the analyst. After drying and cooling, seal and store glassware in a clean environment to prevent the accumulation of dust or other contaminants. Maintain stored glassware capped with combusted aluminum foil.

The following glassware is needed to perform the sediment extraction and purification procedure:

Brown Amber Bottles: 250 ml with Teflon cap (Savillex, 24mm).

Drying Column: 20 mm ID Pyrex chromatographic column with glass wool at bottom and Teflon Stopcock, or Pyrex glass funnel.

Concentrator Tube: Kuderna-Danish - 25 ml, graduated. Ground glass stoppers are used to prevent evaporation of extracts.

Snyder Column: Kuderna-Danish - 3 ball column.

Evaporative Flask: Kuderna-Danish - 250 and 500 ml flat bottom flask.

Centrifuge Tubes: Corex 50 ml with Teflon-lined screw caps.

Micro Reaction Vessels: 1.0 ml or 2.0 ml autosampler vials with crimp cap septa.

Chromatographic Column: 300 mm x 10 mm ID, with Pyrex glass wool at bottom and Teflon stopcock.

Water Bath: heated to 60-70°C.

Graduated Cylinder: 1 or 2 l.

Disposable Glass Pasteur Pipets: 1 and 3 ml.

Syringes: 10 or 25 µl.

Teflon Boiling Chips: Solvent extracted.

Vials: 1 ml to 7 ml glass vials with Teflon-lined caps.

Gas Evaporation Unit: Nitrogen

Note: Volumetric glassware for sample measurement or introduction of internal standards must be calibrated.

4.2 Reagents

Reagent Water: Water containing no analytes above the method detection limit (i.e., HPLC water).

Sodium Sulfate: (ACS) Granular, anhydrous (purified by heating at 400°C for 4 hours in a shallow tray, or other suitable method).

Solvents: Methanol (for rinsing), methylene chloride, hexane, pentane (pesticide quality or equivalent).

Tropolone: Aldrich t8, 970-2, purity 98%

6N HCl

Hexylmagnesium Bromide: 0.5M in diethylether.

Alumina Oxide: Basic Brockmann 1, standard grade 150 mesh Aldrich 19, 744-3 or equivalent. Combust 4 hours at 400°C. Store at 120°C prior to use.

Silica Gel: Grade 923, 100-200 mesh Aldrich 21,447-7 or equivalent. Store at 170°C before use.

Surrogate Spiking Solutions: Refer to GERG SOP-9013 for preparation of appropriate surrogate spiking solutions.

Matrix Spike Standard: Refer to GERG SOP-9013 for preparation of appropriate matrix spiking solutions.

Internal Standard Solution: Refer to GERG SOP-9013 for preparation of appropriate internal standard spiking solutions.

5.0 PROCEDURE

5.1 Sample Extraction

5.1.1 Weigh 20 grams of freeze dried sediment into a 250 ml brown amber bottle. Add the appropriate amount of surrogate standards (~10x MDL) to all samples, spikes, and blanks as described in GERG SOP 9013.

5.1.2 Add 100 ml 0.2% tropolone in methylene chloride to the brown amber bottle, seal with a Teflon screw cap and roll on a roller table for 3 hour. If necessary, centrifuge the sample and decant the organic phase into a 500 ml flat bottom flask.

5.1.3 Repeat step 5.1.2.

5.1.4 Serially transfer the extract to a 50 ml centrifuge tube. Add a boiling chip and concentrate the extract to 4-10 ml. Then add 30 ml of hexane and evaporate to 20 ml at which point only hexane should remain.

5.2 Hexylation

Take extreme care when handling Grignard reagents.

5.2.1 Purge the sample extract in 20 ml at hexane with nitrogen and add 2 ml of hexylmagnesium bromide (2M; Grignard reagent) and seal with a teflon lined screw cap.

5.2.2 Conduct the hexylation reaction in a 70°C water bath for 6 hours.

5.2.3 Allow reaction mixture to cool.

5.2.4 Neutralize excess Grignard reagent by adding 5 ml of 6N HCl.

5.2.5 Shake sample vigorously and allow phases to separate.

5.2.6 Decant organic phase (top) with a pasture pipet into a 125 ml flat bottom flask.

5.2.7 Add 15 ml of a mixture of 3:1 of pentane:CH₂Cl₂ to the aqueous phase in the 50 ml centrifuge tube, shake vigorously, allow phase to separate and decant the pentane:CH₂Cl₂ into the 125 ml flat bottom flask.

5.2.8 Repeat step 5.2.7.

5.2.9 Dry sample with Na₂SO₄

5.2.10 Attach a Snyder column to the 125 ml flat bottom flask containing the combined organic phases and concentrate to 1-10 ml in a water bath (60-70°C). Further concentrate the sample to 2 ml in a concentrator tube.

5.3 SILICA/ALUMINA COLUMN CLEANUP

5.3.1 Fill the glass chromatographic column to about 20 cm with hexane. Weigh 10.0 g of silica and add the silica to the column. Gently tap the column to evenly distribute the alumina. Alternatively, a slurry of alumina in pentane may be used to pack the column.

5.3.2 Allow the silica to settle and then add 10 g of alumina to the top of the silica.

5.3.4 Drain the pentane through the column until the head of the liquid in the column is just above the top of the column. Close the stopcock to stop solvent flow.

5.3.5 Transfer the hexylated sample extract in 2 ml of hexane onto the column. Rinse the extract vial with 1 ml pentane and add it to the column.

5.3.6 Add 50 ml of hexane and elute at a flow rate of ~2 ml/min. Collect the effluent in a 250 ml flat bottom flask.

5.3.7 Concentrate the extract 0.5 to 1 ml.

6.0 QUALITY CONTROL

Quality control samples are processed in a manner identical to actual samples.

6.1 Run a method blank with every 12 samples, or with every sample set, whichever is more frequent. Blank levels should be no more than 3x the method detection limit (MDL). If blank levels for any component are above 3x MDL, samples analyzed in that sample set should be re-extracted and reanalyzed. If insufficient sample is available for re-extraction, the data will be reported and appropriately qualified.

6.2 Run matrix spike/matrix spike duplicate (MS/MSD) samples with every 12 samples, or with every sample set, whichever is more frequent. The appropriate spiking level is 10x the MDL.

6.3 Spike surrogate materials into every sample and QC sample. The appropriate spiking level is 10x the MDL.

6.4 Surrogate and matrix spike recovery acceptance criteria are described in detail in GERG SOP-9013.

6.5 Reference Materials: Reference materials are analyzed when available.

7.0 REPORTING AND PERFORMANCE CRITERIA

7.1 Reporting units are ng Sn/g.

7.2 The minimum performance standard for the method is detection of 5 ng Sn/g for individual butyltins.

Note: The effective minimum performance standard can be adjusted by decreasing final sample volume, increasing sample amount and/or increasing volume injected on the GC-FPD.

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**EXTRACTION OF BIOLOGICAL TISSUES FOR
BUTYLTIN ANALYSIS**

Written by

Terry L. Wade

This document presents the protocol, materials, and quality control used in the performance of the above analysis.

Laboratory Manager
Terry L. Wade

Date

Quality Control Officer
Thomas J. Jackson

Date

Rev. 2

Approved

Mable C. Kinnick 5/27/92

May 27, 1992

**EXTRACTION OF BIOLOGICAL TISSUES FOR
BUTYLTIN ANALYSIS**

1.0 INTRODUCTION

Assessment of the environmental impact of butyltins requires their measurement in tissues at trace levels (parts per billion to parts per trillion).

This standard operating procedure provides a precise and accurate method to quantitatively determine butyltin compounds in tissues. Tissue samples are serially extracted with 0.2% tropolone in methylene chloride using a Tissumizer. The extracts are then hexylated with Grignard reagent. The hexylated extract is dried and concentrated. A silica/alumina column cleanup step is used before the instrumental analysis to remove matrix interferences. The protocol is designed for 15 gram samples. The extract is then submitted for analysis of butyltins by GERG SOP-9013.

2.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

2.1 Sample Collection

Collect tissue samples in combusted glass jars and freeze (-20°C) in the field.

2.2 Sample Preservation and Storage

Store tissue samples at -20°C in the dark. Tissue samples should be shipped frozen to the laboratory and stored at -20°C in the dark until analysis. After subsampling, store excess sample at -20°C in the dark. Store sample extracts in the dark at 4°C.

3.0 INTERFERENCES

Method interferences may be caused by lipid materials or other contaminants in solvents, reagent, glassware, and other sample processing hardware that lead to false positives in GC/FPD detection. All materials used in this method are routinely demonstrated to be free from interferences by

processing procedural blanks identical to samples (one blank per 12 samples or each batch, whichever is more frequent).

Take care in dissecting tissue from animals with oiled exteriors. If possible, rinse oily material from the shells or exoskeleton prior to thawing and removal of tissue for analysis.

Matrix interferences may be caused by compounds other than the analytes of interest that are coextracted from the sample. Biogenic materials that cause interferences in the analysis of tissue extracts are removed prior to GC/FPD analysis by silica/alumina purification.

4.0 APPARATUS AND MATERIALS

4.1 Labware and Apparatus

Clean glassware by water washing with detergent (micro cleaning solution) and rinsing with tap water. Then combust the glassware in a muffle furnace at 400°C for at least 4 hours. Solvent rinses of methanol to dry followed by methylene chloride may be substituted for the muffle furnace heating when determined to be appropriate by the analyst. After drying and cooling, seal and store glassware in a clean environment to prevent the accumulation of dust or other contaminants. Maintain stored glassware capped with combusted aluminum foil.

The following labware is needed to perform the tissue digestion and purification procedure:

Stainless Steel Knife or Shears: For dissecting animals.

Glass Centrifuge Bottles: 500 ml capacity.

Centrifuge Tubes: Corex 50 ml with Teflon-lined screw caps.

Disposable Glass Pasteur Pipets: 1 and 3 ml.

Boiling Chips: Solvent extracted, Teflon.

Water Bath: Heated to 60°-70°C.

Vials and Teflon Lined Caps: 1 ml to 7 ml capacity.

Gas Evaporation Unit: Nitrogen

Tissumizers: Tekmar; Polytron homogenizer, or equivalent.

Flat Bottom Flasks: 125 ml and 500 ml capacity.

Snyder Column: Kuderna-Danish, 3-ball column.

Concentrator Tubes: Kuderna-Danish 25 ml, graduated with ground glass stoppers.

Erlenmeyer Flask: Various sizes.

Microliter Syringes: 1000, 500, 100, 50 and 10 μ l capacity.

Balance: Top loading with an accuracy of 0.01 g.

Electrobalance: Cahn or equivalent, with an accuracy of 0.0001 mg.

Pyrex Glass Wool: Combusted to 400°C.

Chromatographic Column: 300 mm x 10 mm, with Pyrex glass wool plug at bottom and Teflon stopcock.

Microreaction Vessels: 2.0 ml or 1.0 ml autosampler vials with crimp cap septa.

Note: Volumetric glassware used for sample measurement or introduction of internal standards must be calibrated.

4.2 Solvents and Reagents

The procedure requires the following:

Reagent Water: Water containing no analytes above the method detection limit (i.e., HPLC water).

Solvents: Methylene chloride, hexane, pentane (pesticide quality or equivalent).

Tropolone: Aldrich T8, 970-2, purity 98%

6N HCl

Sodium Sulfate: (ACS) Granular, anhydrous (purified by heating at 400°C for 4 hours in a shallow tray or other suitable method).

Hexylmagnesium Bromide: 0.5M in diethylether.

Alumina Oxide: Basic Brockmann 1, standard grade 150 mesh Aldrich 19,744-3 or equivalent. Combust 4 hours at 400°C. Store at 120°C prior to use.

Silica Gel: Grade 923, 100-200 mesh Aldrich 21,447-7 or equivalent. Store at 170°C for at least 16 hours before use.

Surrogate Spiking Solutions: Refer to GERG SOP-9013 for preparation of appropriate surrogate spiking solution.

Matrix Spike Standard: Refer to GERG SOP-9013 for preparation of appropriate matrix spiking solution.

Internal Standard Solution: Refer to GERG SOP-9013 for preparation of appropriate internal standard spiking solution.

5.0 PROCEDURES

Mechanically macerate all tissue samples prior to extraction. Add surrogates after maceration and subsampling, but prior to digestion and extraction.

5.1 Preparation of Samples

5.1.1 Fish - While still partially frozen, rinse fish with reagent water if necessary to remove extraneous material. Dissect the edible portions of the fish or other target organs under contaminant free conditions. Pool sufficient tissue in a combusted mason jar and macerated using a Tissumizer or Polytron blender. Weigh the macerated tissue into a centrifuge bottle (2-15 g wet weight).

5.1.2 Crabs, Sea Urchins, Brittle Stars - Rinse the animal(s) with reagent water if necessary to remove extraneous material. Dissect the tissue of interest under contaminant-free conditions. Pool sufficient tissue in a combusted mason jar and macerated using a Tissumizer or Polytron blender. Weigh the macerated tissue into a centrifuge bottle (2-15 g wet weight).

5.1.3 Clams, Mussels, Oysters - Rinse bivalves with reagent water, if necessary, to remove extraneous material. Shuck bivalves with a stainless steel knife and remove animal from shell. Pool sufficient tissue in a combusted mason jar and macerate using a Tissumizer or Polytron blender. Weight the macerated tissue into a centrifuge bottle (2-15 g wet weight).

5.1.4 Percent Moisture Determination - Remove a separate 5 g aliquot of macerated tissue, place it in a tared weighing pan, and weigh. Dry the tissue at 50°C, allow to cool and then reweigh.

5.2 Digestion and Extraction

5.2.1 Place the tissue in a 200 ml centrifuge bottle. To each sample, add 100 ml 0.2% tropolone in CH_2Cl_2 , 50 g Na_2SO_4 and the appropriate amount of surrogates as described in GERG SOP-9013. Macerate the tissue for 3 minutes with the Tissumizer. Decant the CH_2Cl_2 into a 500 ml flat bottom flask (centrifuge at 2000 rpm, if necessary).

5.2.2 Repeat the extraction two more times with 100 ml aliquots of 0.2% tropolone in CH_2Cl_2 . Combine the CH_2Cl_2 aliquots in the 500 ml flat bottom flask.

5.2.3 Attach a 3-ball Snyder column to the 500 ml flat bottom flask. Add one clean boiling chip. Place the apparatus in a hot water bath (60-70°C) and concentrate to 10-20 ml.

5.2.4 Transfer the sample to a 50 ml test tube. Rinse the 500 ml flat bottom flask with 5-10 ml of hexane and add to test tube. Concentrate to 1 ml in a water bath (60-70°C).

5.3 Hexylation

Take extreme care when handling Grignard reagents.

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5.3.1 Purge the sample extract in 20 ml of hexane with nitrogen, and add 2 ml of hexylmagnesium bromide (2M; Grignard reagent) and seal with a teflon-lined screw cap.

5.3.2 Conduct the hexylation reaction in a 70°C water bath for 6 hours.

5.3.3 Allow reaction mixture to cool.

5.3.4 Neutralize the excess Grignard reagent by adding 5 ml of 6N HCl.

5.3.5 Shake sample vigorously and allow phases to separate.

5.3.6 Decant organic phase (top) with a pasture pipet into a 125 ml flat bottom flask.

5.3.7 Add 15 ml of of a 3:1 mixture of pentane:CH₂Cl₂ to the aqueous phase in the 50 ml centrifuge tube, shake vigorously, allow phase to separate and decant the pentane:CH₂Cl₂ into the 125 ml flat bottom flask.

5.3.8 Repeat step 5.3.7.

5.3.9 Dry the sample with Na₂SO₄

5.3.10 Attach a Snyder column to the 125 ml flat bottom flask containing the combined organic phases and concentrate to 1-10 ml in a water bath (60-70°C). Further concentrate the sample to 2 ml in a concentrator tube.

5.4 SILICA/ALUMINA COLUMN CLEANUP

5.4.1 Fill the glass chromatographic column to about 20 cm with pentane. Weigh 10.0 g of silica and add the silica to the column. Gently tap the column to evenly distribute the alumina. Alternatively, a slurry of alumina in pentane may be used to pack the column.

5.4.2 Allow the silica to settle and then add 10 g of alumina to the top of the silica.

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5.4.3 Drain the pentane through the column until the head of the liquid in the column is just above the top of the column. Close the stopcock to stop solvent flow.

5.4.4 Transfer the hexylated sample extract in 2 ml of hexane onto the column. Rinse the extract vial with 1 ml pentane and add it to the column.

5.4.5 Add 50 ml of pentane and elute at a flow rate of ~2 ml/min. Collect the effluent in a 250 ml flat bottom flask.

5.4.6 Concentrate the extract 0.5 to 1 ml.

5.5 Preparation for Instrumental Analysis

5.5.1 Analyze the samples for butyltins by GERG SOP-9013.

6.0 QUALITY CONTROL

Quality control samples are processed in a manner identical to actual samples.

6.1 Run a method blank with every 12 samples, or with every sample set, whichever is more frequent. Blank levels should be no more than 3x the method detection limit (MDL). If blank levels for any component are above 3x MDL, samples analyzed in that sample set should be re-extracted and reanalyzed. If insufficient sample is available for re-extraction, the data will be reported and appropriately qualified.

6.2 Run matrix spike/matrix spike duplicate (MS/MSD) samples with every 12 samples, or with every sample set, whichever is more frequent. The appropriate spiking level is 10x the MDL.

6.3 Spike surrogate materials into every sample and QC sample. The appropriate spiking level is 10x the MDL.

6.4 Surrogate and matrix spike recovery acceptance criteria are described in detail in GERG SOP-9013.

6.5 Reference Materials: Reference materials are analyzed when available.

7.0 REPORTING AND PERFORMANCE CRITERIA

7.1 Reporting units are ng Sn/g.

7.2 The minimum performance standard for the method is detection of 5 ng Sn/g for individual butyltins.

Note: The effective minimum performance standard can be adjusted by decreasing final sample volume, increasing sample amount and/or increasing volume injected on the GC-FPD.

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SOP-9013

QUANTITATIVE DETERMINATION OF BUTYL TIN

Written by

Terry L. Wade

This document presents the protocol, materials, and quality control used in the performance of the above analysis.

Laboratory Manager
Terry L. Wade

Date

Quality Control Officer
Thomas J. Jackson

Date

Rev. 2

Approved

Mable C. Kennicut #7 5/27/92

May 27, 1992

QUANTITATIVE DETERMINATION OF BUTYLTINS

1.0 INTRODUCTION

The quantitative method described in this document determines butyltins in extracts of water, sediments and tissues. The method is based on high resolution, capillary gas chromatography using flame photometric detection (GC/FPD). This method quantitatively determines tetrabutyltin (4BT), tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT).

Extracts should be prepared as described in GERG SOP's-9010, 9011 and 9012 for water, sediment and biological tissues, respectively.

Sample collection, preservation and storage times are discussed under the analytical procedures for sample extraction and purification.

2.0 APPARATUS AND MATERIALS

A gas chromatograph with a split/splitless injection system, capillary column capability and a flame photometric detector (FPD) equipped with a tin selective 610 nm filter. The output from the detector is collected and processed by an automated HP-LAS 3357 data acquisition software package.

2.1 GC Column

Use a 30-m long x 0.32-mm I.D. fused silica capillary column with DB-5 bonded phase (J&W Scientific or equivalent).

2.2 Autosampler

The autosampler is capable of making 1-4 μ l injections.

3.0 REAGENTS

3.1 Calibration Solution

The calibration solution is comprised of 4BT, TBT, DBT and MBT.

Prepare calibration standards in the concentration range of 1.25 to 50 ng Sn/ μ l (at five concentrations) at a minimum. Add internal standard and surrogate compounds at 20 ng Sn/ μ l to all calibration standards.

3.2 Surrogate Spiking Solution

The surrogate compound for all sample types is tripropyltin chloride. Make the surrogate solution by weighing an appropriate amount of pure material into a volumetric flask and dilute to volume with methylene chloride. Add the surrogates to each sample at a concentration of ~10x the MDL (i.e., 100 μ l of 20 ng Sn/ μ l to a final volume of 1 ml).

3.3 Internal Standard Solution

The internal standard for this analysis is tetrapropyltin. Make the internal standard solution by weighing an appropriate amount of pure material into a volumetric flask and dilute to volume with methylene chloride. Add the internal standard to each sample extract to obtain a final concentration of approximately 2 ng Sn/ μ l. For higher concentrations of oil the internal standard concentration is appropriately increased.

3.4 Matrix Recovery Spiking Solution

The matrix spiking solution consists of 4BT, TBT, DBT and MBT.

Add the matrix spike to samples at a concentration ~10x the MDL.

3.5 Retention Index Solution

Use the calibration mixture as a retention index solution.

4.0 PROCEDURE

4.1 Sample Extraction and Purification

Water samples are extracted and purified (optional) following GERG SOP-9010. Sediment samples are extracted and purified following GERG SOP-9011. Tissue samples are extracted and purified following GERG SOP-9012.

4.2 High Resolution GC-FPD Analysis

4.2.1 GC Conditions

For the analysis of butyltins, the analytical system, or its equivalent, should include at a minimum:

Instrument:	Hewlett-Packard 5880A or HP 5890 Gas Chromatograph
Features:	Split/splitless capillary inlet system, HP-1000 LAS 3357 data acquisition system
Inlet:	Splitless
Detector:	Flame photometric, 610 nm filter
Column:	0.32-mm I.D. x 30-m DB-5 fused silica capillary column (J&W Scientific)
Gases:	
Carrier:	Helium 2 ml/min.
Make-Up:	Helium 33 ml/min.
Detector:	Air 100 ml/min. Hydrogen 80 ml/min.
Temperatures:	
Injection port:	300°C
Detector:	225°C
Oven Program:	60°C to 300°C, hold 10 min.

The GC oven temperature program may be modified to improve resolution.

Daily Calibration:	Mid-level calibration solution; Retention index solution
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(20 µg/ml)

Quantification: Internal standard/calibration

Note: The GC must be capable of the baseline resolution of all target compounds surrogates, and internal standards from each other and from interfering compounds.

4.2.2 Calibration

Prior to every sample set perform the GC calibration at a minimum of three concentrations. One of the concentration levels is near, but above the MDL. The remaining concentrations correspond to the expected range of the sample analytes. A concentration range of 1.25 to 50 ng Sn/µl is recommended. The detector is linear within this range. An average calibration factor from the authentic standard of each individual compound is used to calculate sample analyte concentrations. The initial calibration is verified by the measurement of calibration standards after at least every 12 samples. When possible, the RF for each individual butyltin is calculated from authentic material.

Analyze a mid-level standard immediately prior to conducting any analyses, and after each group of 12 samples. The response factor criteria for an in control calibration check is ±30%.

4.2.3 Retention Time Windows

Retention time windows are established and maintained according to procedures outlined in EPA Method 8000, Section 7.5. Three times the standard deviation of the retention time determined from the calibration solutions is used to calculate a window size.

4.2.4 Sample Analysis

If the instrument is in calibration initiate butyltin analyses with a calibration check, followed by 12 samples, and ending with a calibration check (Table 1). If the response factor for any analyte in the calibration check fails to meet the criteria established in Section 4.2.2, the instrument is recalibrated. All samples that were injected after the standard exceeded the criteria must be reinjected or recalculated based on the analysts review of the data.

Sample injections of 1 to 4 μ l are made with an autosampling device.

If the response for any peak exceeds the highest calibration solution, dilute and reanalyze the extract.

Table 1. Minimum Sample Distribution to Meet QA Requirements During a Typical TBT Analysis.

Sample No.	Description	Function
1	Solvent Blank	Establish instrument background
2	Cal Ck*	Confirm "in calibration" condition
3	Sample #1	Unknown Analysis
4	Sample #2	Unknown Analysis
5	Sample #3	Unknown Analysis
6	Sample #4	Unknown Analysis
7	Sample #5	Unknown Analysis
8	Sample #6	Unknown Analysis
9	Sample #7	Unknown Analysis
10	Sample #8	Unknown Analysis
11	Sample #9	Unknown Analysis
12	Sample #10 (duplicate)**	Unknown Analysis
13	System Blank	Confirm method blank
14	Spiked Blank/Fortified Sample/SRM	Complete Analytical QA***
15	Cal Ck	Confirm "in calibration" condition

- * Criteria $\pm 30\%$ an individual analyte
** 10% of samples analyzed in duplicate
*** Criteria $\pm 30\%$ for all analytes

4.2.5 Calculations

Calculations are based on the methods of internal standards. The general formula for calculating butyltins is found in Section 7.8.2 of EPA SW-846 Method 8000. See Section 6.1 of this method for details of the calculations. This method is modified in that all analyte concentrations are corrected for the surrogate recoveries and all concentrations are reported as μ g Sn/l or g.

5.0 QUALITY ASSURANCE/QUALITY CONTROL (QA/QC) REQUIREMENTS

5.1 Initial Calibration and Continuing Calibration Checks

Prior to the analyses, a three-point response factor calibration curve establishes the linear range of the detector.

Analyze each calibration standard and calculate the response factor (RF) of each compound at each concentration level from the area of the peak and the calculated Sn concentration.

Use the following formula to calculate the response factors of the internal standard relative to each of the calibration standards.

$$RF = (A_s C_{is}) / (A_{is} C_s)$$

where:

A_s = Area for the analyte to be measured.

A_{is} = Area for the internal standard (tetrapropyltin).

C_{is} = Concentration of the internal standard (ng/ μ l)

C_s = Concentration of the analyte to be measured (ng/ μ l).

For every 12 sample analyses or at least once daily, determine the response factor for each compound of interest relative to the internal standard.

Compare the daily response factor for each compound to the initial calibration curve. Calculate the percent difference using the following equation:

$$\text{Percent Difference} = \frac{RFI - RFC}{RFI} \times 100$$

where:

RFI = Average response factor from initial calibration.

RFC = Response factor from current verification check standard.

If the average daily response factors for all analytes is within ± 15 percent of the corresponding calibration curve value, the analysis may proceed. If, for any individual analyte, the daily response factor exceeds ± 30 percent of the corresponding calibration curve value, a three-point calibration curve must be repeated for that compound prior to the analysis of the samples. All samples are calculated from the initial calibration.

5.2 Method Blank Analysis

An acceptable method blank analysis does not contain any target compound at concentration 3 times greater than the MDL. If the method blank does not meet these criteria, the analytical system is out of control and the source of the contamination must be investigated, corrective measures taken, and documented before further sample analysis proceeds.

5.3 Surrogate Compound Analysis

Spike all samples and quality control samples with TPT. Spike the surrogate solution into the sample prior to extraction to measure individual sample matrix effects associated with sample preparation and analysis.

The laboratory will take corrective action whenever the recovery of the surrogate is below 30 percent for water, sediment and tissue matrices.

Take the following corrective action when an out of control event occurs:

- a. Check calculations to assure that no errors have been made.
- b. Check the internal standard and surrogate solutions for degradation, contamination, etc., and check instrument performance is checked.
- c. If the surrogate could not be measured because the sample required a dilution, no corrective action is required. The surrogate recovery is properly annotated.
- d. If the steps above fail to reveal a problem, reanalyze the sample or extract. If reanalysis of the extract yields surrogate recoveries within the stated limits, then report the reanalysis data. If upon reinjection QA criteria are still violated, the sample will be submitted for re-extraction if sufficient sample is available. If the sample was

completely consumed, the data will be reported but designated as outside the QA criteria.

5.4 Matrix Spike Analysis

The laboratory spikes and analyzes a matrix spike every 12 samples or with every sample set, whichever is more frequent. A sample is randomly chosen, split into two subsamples and the subsample is fortified with the matrix spike. The acceptable matrix spike recovery criteria for water, sediment and tissue analysis are:

- The acceptable average recoveries are $90 \pm 20\%$ for 4BT, TBT, DBT and $60 \pm 25\%$ for MBT.

If the matrix spike criteria are not met, reinject the matrix spike on the GC. If the reinjected matrix spike analysis meets the criteria, then report the reanalysis data. If not, submit the entire batch of samples for re-extraction if sufficient sample is available. If the sample was completely consumed, report the data but designate as outside the QA criteria.

5.5 Method Detection Limit

The method detection limit is determined following the procedures outlined in Federal Register (1984), Vol. 49, No. 209: 198- 199.

5.6 GC Resolution

The target compounds, and internal standard must be resolved from one another and from interfering compounds.

5.7 Reference Sample Analysis

When available, analyze reference material. Analyze one sample per batch of samples, and use the results to establish laboratory QC charts. The result should agree within $\pm 30\%$ of the mean of the previously reported data. Use the data produced to construct control charts.

6.0 CALCULATIONS

6.1 Butyltin Calculations

All calculations are based on the methods of internal standards from Section 7.8.2 of EPA SW-846 Method 8000 with the modification that all values are corrected for surrogate recoveries:

- RF = average of $(A_s \times C_{is}) / (A_{is} \times C_s)$

where:

A_s = Area of analyte to be measured

A_{is} = Area of internal standard tetrapropyltin

C_{is} = Concentration of the internal standard tetrapropyltin
(ng Sn/ μ l)

C_s = Concentration of the analyte to be measured (ng Sn/ μ l).

$$C_e = \frac{(A_s)(I_s)}{(A_{is})(RF)}$$

where:

C_e = Sample extract concentration (ng Sn/ μ l).

A_s = Area of the analyte to be measured.

A_{is} = Area of the the internal standard (tetrapropyltin).

I_s = Amount of internal standard added to each extract divided by the final extract volume (V_e).

The actual sample concentration (C) for each compound is calculated by the following formula:

$$C = (C_e) \times \frac{V_e}{V_s}$$

where:

C = Concentration in sample (ng Sn/l or g).

V_E = The final extract volume (ml).

V_s = The original volume of sample extracted (l or g).

Correct the calculated value to one hundred percent recovery based on the surrogate recovery.

6.3 Calculation Notes

6.3.1 To each sample, add a specific amount of surrogate. Monitor the recovery of this surrogate in each sample using the response of the I.S. that is added to the final extract.

$$\text{Percent SUR recovery} = (A_{\text{SUR}} \times C_{\text{IS}}) / (C_{\text{SUR}} \times A_{\text{IS}} \times \text{RF}_{\text{SUR}})$$

where:

A_{IS} = Area of tetrapropyltin

A_{SUR} = Area of tripropyltin.

C_{SUR} = ng Sn in tripropyltin added to
the sample

C_{IS} = ng Sn in tetrapropyltin added to the sample extract

RF_{SUR} = Response factor for tripropyltin.

Correct all analyte concentrations for surrogate recoveries.

7.0 REPORTING

7.1 Reporting Units

Report units in ng Sn/L for water and ng/g for sediments and biological tissue .

7.2 Minimum Method Performance Criteria

The minimum method performance standard for water is detection of 5 ng Sn/L for butyltins. Criteria for sediments and tissues is 5ng Sn/g.

7.3 Significant Figures

Report results to three (3) significant figures.

7.4 Surrogate Recovery

Report surrogate recoveries for every sample analyzed.

7.5 Matrix Spike

Report matrix spike recoveries for each batch of samples analyzed.

7.6 Reference Materials

When available, report the results of the analysis standard reference materials for each batch of samples analyzed.

Note: The effective minimum performance standard can be adjusted by decreasing final sample volume, increasing sample amount and/or increasing volume injected on the GC-FPD.

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I. OBJECTIVE

Collection of subtidal sediments (cores and bulk) from research vessels for chemical and toxicological analysis.

II. NECESSARY MATERIALS AND EQUIPMENT

The materials and equipment required or available for sediment sampling may vary depending on the vessel used for sampling. The investigator should determine the deployment capabilities of the vessel to be used and the equipment available prior to planning the sampling event. The Smith-MacIntyre or Van-Veen grab may be deployed from a vessel with suitable winches, and an A-Frame or Davit arrangement. The Nemesis is best operated with an A-frame. The freeze corer and standard piston corer are best operated from the URI pontoon boat with a removable hatchway in the mid-ship area.

Additional equipment needed:

- Freeze corer 2.0 m long, 20 cm width
- Nemesis 1.3 m long 7.5 cm diameter piston corer
- standard piston corer 1m long, 10 cm diameter
- 3 m long extension rods for standard piston corer
- Smith-MacIntyre (0.1 m) grab sampler
- Stand - for the grab
- Lead weights - 4 lead weights for Smith-Mac
- Cocking bar for Smith-Mac
- Teflon Coated Van-Veen Grab
- Hard hats
- Face shield or other eye protection
- Respirator - if conditions dictate
- Standard safety equipment (i.e. - first aid kit)
- Tub - into which to dump sediment
- Titanium scoop and titanium sampling tools
- Buckets (2-3) - for rinsing
- Electrical tape
- Duct tape
- Tools - screw drivers, wrenches, hammer, other
- Sample containers - cleaned and acid stripped
- Coolers with ice, or other means of cold storage
- Water-proof sample notebook
- Water-proof markers
- Suitable protective clothing (from weather as well as contaminants)
- Foul weather gear

- Boots
- Exposure suits (orange worksuits) for winter sampling
- Gloves - appropriate gloves for suspected conditions
- Differential GPS for navigation
- Line and stainless cable
- Twine/pocket knife
- Core caps
- Polycarbonate core tubes for piston cores
- Vertical extension box for sampling at 1 cm (or greater) intervals
- Methanol
- Dry ice

Whenever possible, back-up equipment should be carried on board.

III. METHODS

1. The top 1-2 cm of an undisturbed grab sample is sampled using a clean titanium scoop for chemistry and toxicity analysis. Approximately 2-3 Van-Veen or 3-5 Smith Mac grabs are needed to collect a 3-liter composite sample. The surface material is composited in a 12-liter Nalgene bucket, stirred with a titanium stirrer for ~30 seconds, and then subsampled into precleaned containers for organic, inorganic chemistry, and toxicity studies. Rinse the scoop with distilled water, acid, and methanol between grabs. The grab sampler should be "washed-down" with seawater between stations. The samples are stored on ice during collection and at -20°C upon return to the laboratory.
2. Cores are typically taken one of three ways:
 - a. Taking a short core from an undisturbed grab sample
 - 1) Insert "small" (15 cm) cores into the middle of an undisturbed grab sample.
 - 2) Place a cap on the top of the core and remove the core from the grab.
 - 3) Place the second cap on the core after removal from the grab.
 - 4) All caps and liners are precleaned with acid, methanol, and DI. Liners are capped until used.
 - b. Long piston cores

Piston cores are used to take deeper (~1 m) inch cores. A standard piston corer, the biological corer, is used to retrieve cores from intermediate water depths (<20 m). The corer uses polycarbonate tubes and is deployed using a series of 3 meter long extension rods to push the corer into the sediment. Cores up to one meter long are recovered using this design. Biological cores are stored in the vertical position and are transported to the lab for further analysis.

A trigger release piston corer, the Nemesis, is used in deeper water (>10 m). The landing pad design has lead weights loaded at the top and triggers upon contact with the sediment/water interface. The polycarbonate core barrel is then forced into the sediment to recover 1 to 1.3 meter cores from any water depth. The Nemesis cores are stored vertically and transported to the lab for analysis. Both types of piston are sampled by using a vertical extrusion box that extrudes the core out of the top of the core tube in 1 cm (or greater) increments without disturbing the core.

c. Freeze cores

A freeze corer is used to retrieve undisturbed sediments even in flocculent or gaseous sediments. This corer is a two-meter long, stainless steel rectangular box lined with plywood that functions as insulation. The box is filled with a combination of one part methanol and three parts dry ice and creates a freezing surface to which the sediment adheres. The corer is lowered through the water column and then pushed into the sediment column where it remains for 15-20 minutes. During this interval a thickness of 1-4 cm of sediment freezes to the exterior of the box. Frozen slab cores are recovered at lengths from 0.5 to one meter. Cores were wrapped in foil and kept frozen for analysis in the lab. Cores are sectioned using a titanium "hot wire" cutting device in increments of 1 cm (or greater).

IV. TROUBLESHOOTING

When using the Smith-MacIntyre grab in very soft sediments, it may be necessary to attach plywood "shoes" to the landing pads. This is necessary to increase the sensitivity of the "firing" mechanism.

V. STATISTICAL ANALYSIS AND DATA USAGE

Not applicable.

VI. REFERENCES

None.

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I. OBJECTIVE

Obtain an estimate of the availability of heavy metals to benthic organisms by determining the molar ratio of simultaneously extracted metals (SEM) to acid volatile sulfide (AVS), i.e. SEM/AVS. Ratios of 1.0 or greater indicate that heavy metals are likely to be bioavailable; whereas ratios significantly less than 1.0 would indicate that they are not bioavailable.

II. NECESSARY MATERIALS AND EQUIPMENT

- Reaction flasks and traps
- Volumetric flasks
- Burettes
- Pipettes
- Dry nitrogen gas
- SAOB solution - NaOH
- Acetic acid, EOTA
- Thiosulfate titrant
- Starch indicator
- Vanadous chloride stripping solution
- 6M hydrochloric acid
- Sulfide selective probe and meter
- Syringes and needles
- Acid bath
- Filtering apparatus
- Filter paper
- Sample bottles for SEM
- ICP for analysis of SEM
- Mercury analyzer for analysis of Hg

III. METHODS

A. Determination of AVS

The attached method of Boothman is used for the determination of the AVS.

B. Determination of SEM

1. After the generation of sulfide has been completed, the remaining sediment suspension is filtered through a 0.45 μm membrane filter resistant to attack by

acid. The solution is transferred to a volumetric flask and diluted to volume with deionized water.

2. The concentrations of Cd, Cu, Ni, Pb, and Zn are determined by either ICP, or atomic absorption spectrometry.
3. The concentrations of Hg are determined by a mercury analyzer.
4. The concentrations of the SEM in ug/g dry sediment are converted to $\mu\text{mole/g}$ and added.
5. The ratio of SEM/AVS is calculated.

IV. TROUBLE SHOOTING

Discussed in methods section.

V. STATISTICAL ANALYSIS AND DATA USAGE

Not applicable.

VI. REFERENCES

See Boothman attachment.

Acid-volatile sulfide determination in sediments using sulfide-specific electrode detection

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I. Introduction

Acid volatile sulfides (AVS) are amorphous or moderately crystalline metal monosulfides, primarily FeS. They have been hypothesized as the single most important factor controlling the availability of heavy metals to benthic organisms in anoxic sediments (1). The molar ratio of extractable metal to AVS is postulated as an indicator of metal availability. However, methods used to determine AVS have been quite varied in both reagents and conditions. In order to insure that data used by different investigators to test the AVS hypothesis be comparable, a common protocol for the sampling and analysis of AVS needs to be established. The method described follows the findings of Cornwell and Morse (2). Comparisons of this method with a gravimetric method used at Manhattan College have reportedly given equivalent results (D. Hansen, personal communication).

II. Sample collection and storage

The accurate determination of acid volatile sulfides in sediments places a number of rather stringent requirements on the handling of samples after collection. Sulfide ion is thermodynamically unstable in the presence of dissolved oxygen, and so sediments from anoxic environments must be preserved in such a way as to protect any sulfides present from reaction with air. Storage containers must exclude or minimize air space above the sediments; if possible, purging of container headspace with dry, oxygen-free nitrogen gas would be helpful. Sediments should be kept cold or frozen during storage and transportation. Wet sediments may be stored at 4°C for short time, but anoxic sediments stored at 4°C for 20 days show significant changes in metals' partitioning, suggestive of oxidation of the sediment (3). Freezing of sediments seems to cause the least change in the speciation of metals (and by implication sulfides) in anoxic sediments; comparison of metals' extractability in fresh sediments and sediments stored for 20 days at -30°C showed essentially no significant differences. Drying of sediments, either in air or by freeze-drying, has been shown to reduce the concentration of AVS measured in anoxic sediments and should be avoided. The loss of AVS may be due to oxidation or formation of more crystalline (and non-acid volatile) sulfides

III. Determination of Acid Volatile Sulfides

The classification of sulfides as "acid volatile" is an operational definition, that is, the extent to which mineral sulfide phases are volatilized by the analysis will depend on the analytical conditions employed, e.g. acid concentration, time, etc. For AVS data obtained by different investigators to be comparable, the reaction conditions utilized to volatilize sediment sulfides must be similar; the methods used to isolate and quantify the volatilized sulfides, on the other hand, may vary according to instrumental or laboratory availability. In the method presented here, acid volatile sulfides are determined by reaction of sediment sulfides with 1M HCl to form gaseous H₂S and purging the evolved H₂S with nitrogen. The purged H₂S is then trapped in sulfide anti-oxidant buffer (SAOB) (4), diluted to volume and the S²⁻ concentration measured with a sulfide ion-specific electrode (Orion 94-16A). Overall sulfide recovery is determined by analysis of aliquots of a working sulfide standard solution and sediments which have been previously well characterized.

A. Volatilization and trapping of sediment sulfides

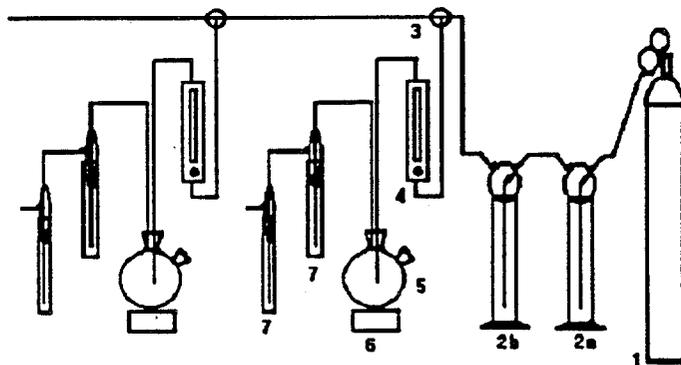


Figure 1. Apparatus for AVS determination: 1. N₂ cylinder; 2. Gas washing bottles: (a) oxygen scrubbing solution, (b) deionized water; 3. Three-way stopcock; 4. Purge flow controller; 5. Reaction flask; 6. Magnetic stirrer; 7. Sulfide traps.

The apparatus used for the volatilization and trapping of acid volatile sulfides in sediments, illustrated in Figure 1, is an adaptation of the system developed by Allen and co-workers at the University of Delaware (5) and DiToro et al. (1). This configuration of the glassware allows the acidification of the sediment while minimizing the entrainment of laboratory air into reaction vessel. It also allows the use of more than one analytical setup at a time while providing purge gas control for each individual analysis.

To prevent oxidation of sulfides due to oxygen in the analytical train, the apparatus should be purged with oxygen-

free nitrogen for at least 30 minutes prior to initiating analyses.

1. Wet sediment (ca. 10 g) is weighed into a 250-ml standard taper round bottom flask.
2. Fifty milliliters (50 ml) of deaerated deionized water (DDIW) is added to cover the sediment, a magnetic stir bar placed in the flask and the flask is placed into the sampling apparatus. Impinger (trap) bottles should be filled with 50 ml of SAOB and 30 ml of DDIW.
3. Initiate purge gas flow at 100 ml min^{-1} to remove any entrained air from the headspace and purge for 10 minutes. Reduce flow to 40 ml min^{-1} .
4. Halt purge gas flow and slowly inject 10 ml of 6M HCl (over approximately 15 sec.) through the septum sidearm, resulting in a concentration of 1.0 M HCl (neglecting the water content of the sediment).
5. Resume purge gas flow of 40 ml min^{-1} and stir sediments vigorously. Purge and trap generated H_2S for desired time (usually 30 minutes).
6. Stop purge flow, rinse impingers with DDIW into bottles and remove bottles from apparatus.

B. Measurement of sulfides by ion-specific electrode

Note: Sulfide electrode and meter should be calibrated prior to performing sediment analyses using sulfide standards prepared in SAOB diluted 1:1 with DDIW.

1. Pour bottle contents into 100-ml volumetric flask. Rinse bottle with DDIW, adding rinse to the vol. flask. Dilute to volume with DDIW.
2. Pour contents of volumetric flask into 150-ml beaker, add magnetic stirring bar and place on stirrer. Begin stirring with minimum agitation to avoid entrainment of air into solution and minimize oxidation of sample during the measurement.
3. Rinse sulfide and reference electrodes into waste container and blot dry with absorbent tissue. Immerse electrodes in sample solution.
4. Allow electrode response to stabilize (8-10 minutes), then take measurement of sulfide concentration (C_s2^-). Reading may be directly in concentration units, if the meter is in concentration mode and a 2-point calibration has been performed, or in millivolts. If the millivolt reading is used, convert millivolts to concentration using the calibration curve obtained from standard solutions.

C. Calculation of AVS concentration in sediments

1. The sediment dry weight/wet weight ratio (R) must be determined separately. Acid volatile sulfides can be oxidized or altered to non-acid volatile forms during various drying processes.

2. AVS concentration in a sample is calculated using the formula:

$$\begin{aligned} \text{AVS}(\mu\text{mol/g dry sediment}) &= \frac{(C_{s2-}) \times V_{\text{trap}}}{\text{g wet sediment} \times R} \\ &= \frac{0.1 \times C_{s2-}}{\text{g wet sediment} \times R} \end{aligned}$$

when $C_{s2-} = \mu\text{mole liter}^{-1}$ (μM) and $V_{\text{trap}} = 100 \text{ ml}$.

D. Calibration of sulfide-specific electrode

1. Direct concentration (2-point calibration)
 - a. select CONC mode on meter.
 - b. press the CAL button on the meter. The CAL1 light should come on.
 - c. immerse electrodes in first calibration standard as for sample (III.B.3-4) and allow response to stabilize. Adjust the concentration displayed to match the standard concentration, using the ↑ and ↓ keys. Press the ENTER key.
 - d. After a few seconds, the CAL1 light should go off and the CAL2 light should come on. Repeat step c for the second calibration standard. After pressing ENTER, the SAMPLE light should come back on, indicating calibration is complete and providing direct readout of concentration.
 - e. Press SLOPE and verify that a value near the theoretical slope (-29.6 mV/decade) is displayed. If the value is far off, repeat the calibration or prepare new standards.
2. multipoint calibration
 - a. select MV mode on meter.
 - b. immerse electrodes in first calibration standard as for sample (III.B.3-4) and allow response to stabilize. Record the electrode response. Repeat for other standards.
 - c. The calibration curve is obtained by linear regression of millivolts against log concentration.
3. Sulfide calibration standards

Calibration standards are prepared from the primary sulfide stock solution (IV.D.). The primary stock concentration must standardized by iodometric titration (IV.D.2) before preparing standards.

 - a. Prepare 700 ml of diluent by mixing 350 ml of SAOB (IV.B) with an equal volume of deaerated deionized water (IV.A).
 - b. Prepare a working stock solution (approx. 1500 μM). Pour 50 ml of the 1:1 SAOB diluent in a 100-ml volumetric flask. Pipette an appropriate volume of the primary stock and an equal volume of SAOB into

be green or blue. Bubble nitrogen through the solution until the color becomes purple. When the solution returns to a blue or green color, the oxygen stripping capacity has been exhausted; it may be replenished by the addition of more amalgamated zinc or a slight amount of conc. HCl.

D. Sulfide stock solution

A sulfide stock solution should be prepared and maintained for use in quality assurance and calibration. An aqueous solution of $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ of sufficient concentration may be used as a stock for secondary stocks for spiking sediments, calibrating, etc. The concentration of this stock should be determined before each use by iodometric titration or other standardization techniques.

1. Preparation

- a. Wash crystals of $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ with deionized water and blot dry.
- b. Weigh approximately 12 g of $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ and dissolve in 900 ml DDIW.
- c. Pour into a 1.00-l volumetric flask and dilute to volume with DDIW.

2. Standardization

- a. Pipette 10.00 ml of standard iodine solution into each of two 125-ml Erlenmeyer flasks.
- b. Pipette 2.000 ml of sulfide stock into one flask. Pipette 2.000 ml of DDIW as a blank into the other flask.
- c. Add 5.00 ml of 6M HCl into each flask, swirl slightly, then cover and place in the dark for 5 minutes.
- d. Titrate each with 0.025N thiosulfate solution, adding soluble starch indicator when the yellow iodine color fades. The end point is reached when the blue color disappears.
- e. The sulfide concentration may be calculated from:

$$\text{Sulfide } (\mu\text{mol/ml}) = \frac{(T_{\text{blank}} - T_{\text{sample}}) \times N_{\text{S}_2\text{O}_3^{2-}}}{V_{\text{sample}}} \times \frac{1 \text{ mole S}^{2-}}{2 \text{ equiv S}^{2-}} \times \frac{1000 \mu\text{moles}}{1 \text{ mmole}}$$

where T = volume of titrant used for the blank and sample (ml)

N = concentration of $\text{S}_2\text{O}_3^{2-}$ titrant
V = volume of sample used (2.00 ml)

E. Standard Iodine solution (0.025N): Dissolve 20-25 g KI in 100 ml deionized water. Weigh 3.2 g I_2 and dissolve in KI solution. Dilute to 1.00-l with deionized water. This solution may be standardized against the thiosulfate solution.

F. Thiosulfate titrant (0.025N) may be purchased commercially or prepared in the laboratory. If prepared in the lab, it should be standardized against potassium dichromate.

1. Preparation: Weigh approx. 6.2 g of $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ into 500-ml beaker. Add 0.1 g Na_2CO_3 and dissolve in 400 ml DDIW. Pour into 1.00-l vol. flask and dilute to volume with DDIW.
2. Standardization
 - a. Weigh 0.02 g dry K_2CrO_7 into a 500-ml Erlenmeyer flask and dissolve in 50 ml deionized water.
 - b. Dissolve 3 g of KI in 50 ml of deionized water, add 5 ml of 6M HCl, and add to KI solution. Swirl, cover and store in dark for 5 minutes. Add 200 ml deionized water and titrate with the thiosulfate solution, adding starch indicator when the yellow iodine color fades, until the blue color fades to pale green.
3. Calculate the thiosulfate concentration as follows:

$$N(\text{S}_2\text{O}_3^{2-}) = \frac{\text{g K}_2\text{CrO}_7}{\text{ml S}_2\text{O}_3^{2-}} \times \frac{1 \text{ mole K}_2\text{CrO}_7}{294.19 \text{ g K}_2\text{CrO}_7} \times \frac{6 \text{ equiv K}_2\text{CrO}_7}{1 \text{ mole K}_2\text{CrO}_7} \times \frac{1000 \text{ ml}}{1 \text{ l}}$$

G. Soluble starch indicator is prepared by dissolving 1.0 g starch in 100 ml boiling deionized water.

V. Preparation of sulfide electrode and meter

The sulfide and reference electrodes and meter should be used and maintained as per the manufacturer's specifications. The instructions below give the brief description; see the meter or electrode operating manuals for detailed instructions.

1. Clean the inner sleeve of the double junction reference electrode with deionized water and fill the inner sleeve with the appropriate filling solution (saturated AgCl).
2. Clean and dry the outer sleeve walls with deionized water. Moisten the gasket of the outer sleeve with the outer sleeve filling solution (10% KNO_3), slide the sleeve on over the inner sleeve and screw on the end cap. Fill the outer sleeve with filling solution.
3. Inspect the surface of the sulfide electrode and polish if necessary (see electrode operating instructions for details).
4. Connect the electrodes to the meter.

VI. References

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I. OBJECTIVE

To determine the percentages by weight of sand, silt, and clay in sediment samples.

II. NECESSARY MATERIALS AND EQUIPMENT

- 4-5 g sediment
- Pre-weighed 250 ml beakers
- Source of deionized water
- 0.063 mm sieve, 5-inch diameter
- 0.025 mm sieve, 3-inch diameter
- Scale capable of weighing to 0.0001 g
- Laboratory oven (100°C)
- Dispersant pyrophosphate (4%)
- 1 N acetic acid
- Hydrogen peroxide (30%)
- Elzone Model 180XY particle size analyzer

III. METHODS

A. Preparation of Samples

1. Obtain 4-5 grams of each sample and place in 250 mL polyethelene beakers. If samples are frozen, let them thaw out.

Removal of Carbonates

2. Add enough 1N Acetic Acid to cover the sample completely, cover and shake well to mix thoroughly. Allow to sit 24 hours under fume hood.
3. Quantitatively transfer samples to 60 mL centrifuge tubes.
4. Centrifuge samples for 10 minutes at 2300 rpm.
5. Pour off liquid into acid drain (in fume hood)
6. Add 50 mL of distilled water to samples and shake well to rinse away acetic acid. Repeat centrifuge and disposal of supernatant under the conditions above.
7. Transfer sample back to 250 mL beakers for the peroxide treatment below (provides more volume for the gases to escape).

Removal of Organics

1. Add 5 mL concentrated hydrogen peroxide (30%) to samples, stir and cover lightly to allow vapors to escape.

2. Allow to sit overnight in fume hood then add another 10 mL to the samples stir and allow to sit for another 24 hours. **If excessive bubbling occurs, squirt a bit of DI H₂O into beaker to slow the reaction.
3. Continue to add hydrogen peroxide in 5-10 mL increments every 24 hours until the reaction no longer occurs (A 1:10 ratio insures that the reaction has gone to completion without H₂O₂ as the limiting reagent, i.e. 5 g sample and 50 mL H₂O₂ (30%).
4. Transfer samples to centrifuge tubes and spin under the conditions above.
5. Decant, rinse with DI H₂O and spin again.
6. SAMPLES READY FOR SIEVING at half the Elzone orifice size.
For 128µm orifice seive at 63µm. For 48µm orifice seive at 25µm.

B. Analyzing Samples

Elzone Pre-Operation

1. Remove beaker from stand by lowering stand with screw. Rinse off orifice tube stirrer and electrode with sodium pyrophosphate (4%). Empty beaker (stored with DI) and add sodium pyrophosphate solution (4%). Empty supply beaker (stored with DI) and add sodium pyrophosphate solution. Make sure that rubber cork on waste flask is sealed tight. Put toggle switch to "count" on Elzone. Replace beaker to original position with metal clip on exterior.

Elzone Operation

2. Turn on Elzone by pushing power button. Turn on the stirrer at a low speed (no cavitation). Put toggle switch at "flush" until glassware is filled and most air bubbles are gone. (Sometimes a large size bubble will be in tube leading from Elzone to waste flask, pinch tubing lightly to remove bubble.) Return toggle switch to "count".
3. Enter start-up display prompts (use [SCAN] keys)
Date: enter numbers for day, month, and year
Operator ID: enter your initials
Select (1-4): enter 1 to get to main display (Note: If you want to enter title headings for your printouts, enter 4 and follow display instructions.)
4. Main Display
 - a. Enter sample number. *Check if status set is one you need .*
 - b. use [SCAN] keys to get to status and enter status set number (Enter 1 for 120µm orifice and 2 for 48 µm orifice).
 - c. use [SET] key to remove cursor
 - d. put toggle switch to "vacuum"
 - e. use [NORM] key to normalize system for the electrolyte, wait for word "normalize" to go away, then push [START] key.

- f. check coinc., it should read 0.000 (if not, empty beaker and put in fresh electrolyte) key [SET] then [Y] then [SET], shake sample thoroughly and put in ultrasonic bath for 30-60 seconds to break up fines and reduce blockage.
 - g. add 2 drops of sample to beaker
 - h. renormalize..repeat steps (d) and (e)
 - i. check coinc., it should be below 0.025 with an ideal coinc about 0.020 (if coinc is too low (below 0.015) add more drops of sample, if coinc is too high, pour a little out, add more electrolyte and renormalize) key [SET] [Y] [SET].
5. a. key [ANLS] to enter analyze display (next screen); set display area to [Ao] for first set of 5 data displays by keying [DSPL] until [Ao] comes up, then key [SET]; key [SCAN] to "status" and enter status set number for the orifice being used and key [SCAN] to "SmplNr" to enter sample number, then key [SCAN] 2^x to return cursor to top
 - b. put toggle switch to "vacuum", then key [START] (ticking sound indicates sample in progress) *if blockage occurs*, switch toggle to "count", push black plunger button 2-3 times, switch toggle back to "vacuum" and then key [START] to resume.
 - c. after analysis stops, the top number reads 12800, (by either peak, total, time, or manual), put toggle to "count"
 - d. use [SCAN] keys to move cursor to "popl" ; key [ALT] to change "popl" to "volm"; key [CONV] to convert histogram to a volume histogram
 - e. key [DSPL] once to change to next display area (Ao, A1, B, Co and C1) and then key [SET]; key [SET] again to return to main display
 - f. lower sample beaker and rinse orifice tube, stirrer and electrode with electrolyte solution
 - g. empty beaker into 20 liter container marked "Na pyro - needs to be refiltered" and rinse out beaker, then add fresh electrolyte solution and place it back on the stand; add more electrolyte solution to supply beaker, if necessary
 - h. put toggle switch to "flush" to remove any air bubbles, then put toggle back to "count"
6. Repeat steps (4) and (5) for each sample.
 7. After running 5 samples (one in each display), send them to the computer to save on disk using PC Talk program.
 8. When finished analyzing samples on the large orifice tube, sieve the samples through the 25 μm sieve for use on the 48 μm orifice.

Blending Section System

1. Turn on Elzone, key [SCAN] to select (1-4): enter 1; key [SET], key [ANLS], then key [DSPL] until you are in C1 Area, key [SET], shut off Elzone (this clears all screens).

2. Turn Elzone back on, enter 1, [SET] [ANLS]; key [DPSL] and [SET] once to enter "Ao" display; this area should be completely empty, if not key [ZERO] [Y].
3. Send low end of sample data (fine) (i.e. sample run on 48 μm orifice from computer disk to Elzone using PC Talk program into display "Ao", key [DSPL] and [SET] to enter "A1".
4. Send high end of sample data (coarse) (i.e. sample run on 120 μm orifice) to "A1".
5. Prepare B256 data area to receive "Ao" and "A1" data.
 - a. key [DSPL] to move to the "B" display, make sure it's a B256 area.
 - b. key [SCAN] to smpl# and enter sample number (for printout)
 - c. key [SCAN] to status and enter status for high end sample (1)
 - d. key [SCAN] back to top
 - e. key [ZERO] [Y], if necessary, and key [RSCL]; enter 1 to rescale area for .707 to 63. Ignore "Bad Log" warning.
6. Key [BLEN]. Use [ALT] to enter "Ao" data. Key [BLEN] again to transfer and rescale data.
7. Extrapolate low end of "Ao" curve (if you're confident that an extrapolation would be accurate).
 - a. key [MARK], key [SCAN] to move low marker to lowest acceptable data pt., key [ALT], key [PEAK] and move high marker to peak of curve, key [MARK] to return to "curs." mode.
 - b. key [BLEN], choose "Ex" using [ALT], key [BLEN] to extrapolate; if satisfied with extrapolation continue to next step, if not, key [WIPE] and repeat step 7.
8. Key [BLEN] and key [ALT] until it reads "A1". Key [BLEN] to rescale and transfer data from the "A1" screen to the combination "B" screen. When this is complete, you should see both of the curves on the same screen and an area of overlap, if the overlap is good, move markers to fine tune (Step 9), if the overlap is not good, key [WIPE] and repeat steps 7 and 8.
9.
 - a. Key [MARK] and note "LMRK" on the left side of the screen. Move lighted markers (one for each curve) from left to right to the first point of intersection/overlap.
 - b. Key [ALT] to switch to "HMRK" and move the markers right to left to the other point of intersection/overlap. Keep markers about 20 channels apart (low $>4 \mu\text{m}$ a
10. Key [BLEN] to combine the two curves into one histogram.
11. After curve is blended save the screen to disk, key [DSPL] to switch to "C1" screen, shut off Elzone. To blend another sample, begin again at step 2. Printing (See shortcut, step 4)

1. Key **[PRINT]**, enter heading numbers. Key **[MARK]**, **[1]**, **[Y]**.
2. To print percentiles:
 - a. key **[PCTL]**
 - b. enter either the percentage or **[SCAN]** once and enter size
 - c. key **[PCTL]**, then key **[Y]** to print
3. To plot graph:
 - a. key **[PLOT]**
 - b. enter headings, key **[MARK]**, then key **[1]**, **[Y]**
 - c. enter **[SKIP]**, and choose 0, 1 or 2:
 - 0 - prints out all channel pts.
 - 1 - prints out every other channel point
 - 2 - prints out every 2nd channel point
4. Shortcut to print all of the above (smooth data 3 times, percents, graph, stats, volume #):

- a. key **[STRG]**, **[R]** and 2

Computer

1. Turn on computer. Put IBM PC Talk 3 -1/2" disk in right side slot - Drive A.
2. Put data disk in left side - Drive B.
3. To format data disk: A> format b: **[ENTER]**
4. To make a directory: A>B: - key B colon to enter "B" drive
 - a. type **["MKDIR file name"]**
 - b. to make a directory within a directory, key **["CD file name"]**
5. To remove a directory:
 - a. enter **["DIRECTORY NAME"]** and **["Del file name"]** The directory must be emptied before it can be deleted, so repeat (a) until directory is empty.
 - b. hit **[CD]** until back to main directory
 - c. hit **[RD "file name"]**, this removes directory
6. When you have entered directory that you want to store data in, go back to "A" drive, i.e. B>A: - type **"PC-Talk" [ENTER]**.
7. To transfer files *from Elzone to computer*:

- a. When ready to transfer files from Elzone to computer key [DSPL] until the first data set area comes up, then key [SET]
 - b. hit [ALT] - [R] on computer and enter file name for disk *DO NOT [ENTER]*
 - c. key [XMIT] on Elzone
 - d. *NOW* key [ENTER] on computer, then key [XMIT] on Elzone again.
 - e. when file is transferred, key [ALT] - [R] to terminate receive command.
8. To transfer file *from computer to Elzone*:
- a. hit [ALT] - [T] on computer and enter file name *DO NOT [ENTER]*
 - b. hit [RECV] on Elzone
 - c. hit [ENTER] key on computer; *note* :display area on Elzone must be completely empty

When finished, hit [ALTX] and then [Y].

Miscellaneous

1. Sieving: Large particle size should be half orifice diameter, i.e. for the 120 μm orifice, use 63 μm sieve to run samples less than 63 μm . Sieve sample through 25 μm sieve to run on 48 μm orifice.
2. Sodium pyrophosphate electrolyte solution preparation: 816.33 g sodium pyrophosphate/20 liters distilled water.

Shutdown of Elzone

1. Empty all beakers and waste flask into container for solution that needs to be refiltered.
 2. Fill sample beaker and supply beaker with distilled water. "Flush" system with distilled water and then Leave rubber cork on waste flask loose.
 3. Shut off Elzone, and put toggle switch to "flush" to drain the tubing.
- C. Calculation of Grain Size Parameters % Sand, % Silt, and % Clay
1. Dry sieved fraction $> 63\mu\text{m}$ at 100°C in drying oven in a pre-weighed 250 ml beaker and reweigh. The weight of this fraction is the dry weight of the sand fraction.
 2. Dry the sieved fraction $> 25\mu\text{m}$ and $< 63\mu\text{m}$, and the sieved fraction $< 25\mu\text{m}$ at 100°C in a drying oven in pre-weighed 250 ml beaker, and weigh both fractions. The sum of the weights of these two fractions is the dry weight of the clay + silt fractions.
 3. Add the dry weight of the sand fraction and the dry weight of the clay + silt fraction to obtain the total dry weight of the sample.
 4. The % sand is calculated by the expression:

$$\frac{\text{dry weight of } > 63\mu\text{m fraction}}{\text{total dry weight of sample}}$$

5. The cumulative volume % grain size distribution of the < 63 μ m fraction has been determined by the Elzone analysis and is stored on disk. The % silt is calculated by the expression:

$$\frac{(4\mu\text{m} < \text{volume \% of } < 63\mu\text{m fraction} < 63\mu\text{m}) \cdot (\text{dry weight of } < 63\mu\text{m fraction})}{\text{total dry weight of sample}}$$

The % clay is calculated by the expression:

$$\frac{(\text{volume \% of } < 63\mu\text{m fraction} < 4\mu\text{m}) \cdot (\text{dry weight of } < 63\mu\text{m fraction})}{\text{total dry weight of sample}}$$

IV. TROUBLE SHOOTING

Discussed in methods section.

V. STATISTICAL ANALYSIS AND DATA USAGE

Not applicable.

VI. REFERENCES

Folk, R.L. 1968. Petrology of Sedimentary Rocks, Herphill Publishers, Austin, TX.

POINT OF CONTACT:

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I. OBJECTIVE

Use low-field susceptibility logs of multiple cores from a study site:

1. Correlate the cores.
2. Evaluate the spatial heterogeneity of sedimentatin at the study site.
3. provide an initial estimate of the age of the core that can be used to design a sampling plan for the core.

II. NECESSARY MATERIALS AND EQUIPMENT

- a. Bartington Instruments MS-2 Susceptibility Meter with RS232 part Loop-Sensor Rings (75mm and 100 mm diameter)
- b. Non-magnetic tracks for core handling
- c. Lap-top IBM - compatible PC
- d. Susceptibility - logging software
- e. Printer
- f. Non-magnetic piston to stabilize sediment-waker interface
- g. Tape measure (metric)
- h. Electrical tape
- i. (DEC 5000 workstation)
- j. CORPAC core correlation software

III. METHODS

1. Drain all water from top of piston core using a siphon hose and insert adjustable non-magnetic piston into to of core tube to stabilize sediment-water interface.
2. Warm-up susceptibility meter and sensor loop for 30 minutes.
3. Initialize software and proceed to log core at depth increment (usually 2-4 cm). Core log will take ~ 20-30 minutes to complete.
4. Plot logs of multiple cores obtained from a site.
5. Transfer data to DEC 500 Workstation and use CORPAC software to correlate susceptibility logs.

IV. TROUBLESHOOTING

Discussed in methods section.

V. STATISTICAL ANALYSIS AND DATA USAGE

Not applicable

VI. REFERENCES

None.

POINT OF CONTACT:

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I. OBJECTIVE

This SOP describes the determination of the organic carbon content of marine or estuarine sediment by the loss-on-ignition method.

II. NECESSARY MATERIALS AND EQUIPMENT

- Muffle furnace with temperature control
- Coors 10cc crucibles
- 1 cc brass volumetric sampler
- Digital balance accurate to .001 gram
- Calibration weights
- Subsampling tools
- Cellulose filter paper
- Large dessicator
- 1 pound dessicant (silica gel)
- Oven gloves
- Oven tongs
- Crucible trays

III. METHODS

Estimation of the organic carbon content is accomplished by first drying a sediment sample, combusting the sample for 1 hour at 550°C, and then determining the weight lost on ignition at 550°C. Multiplication of the weight lost by ignition at 550°C by the factor .44 provides an estimate of the organic carbon content of the sediment sample. Our approach closely follows the method of Dean (1974).

A. Sample Preparation and Analysis

1. Sediments should be thawed and homogenized using an aluminum weighing pan and plastic spatula.
2. A calibrated brass sampler is used to obtain a 1 cc sediment sample and this sample is placed in a previously dried, labeled, and weighed Coors 10 cc crucible.
3. The crucible is re-weighed and the sample weight determined to the nearest milligram.

4. The sample is placed in a muffle furnace that has been pre-heated to 110°C and is maintained at this temperature for 12 hours. Samples are analyzed in batches of 10-20 samples.
5. After 12 hours the samples are removed from the muffle furnace, placed in a dessicator, and allowed to cool to room temperature.
6. The samples are then re-weighed and the weight determined to the nearest milligram. The net loss in sample weight after heating at 110°C for 12 hours is the water content of the sample.
7. The samples are returned to the muffle furnace. The temperature of the muffle furnace is raised to 550°C by first setting the furnace controller at 510°C and after this temperature is attained, increasing the controller setting in 10°C increments until the oven is at 550°C. Care must be taken so that the furnace does not overrun the 550°C setting.
8. Heat the samples for 1 hour at 550°C.
9. Turn off and open the muffle furnace and allow the samples to cool for approximately 2 minutes. Remove the crucible tray holding the samples with the oven tongs and gloves and place them back into the dessicator.
10. Allow the samples to cool to room temperature in the dessicator and then re-weigh them to the nearest milligram.
11. The weight loss between Step 6 and Step 10 is the loss-on-ignition.
12. Multiply the loss-on-ignition weight by .44 and divide by the dry weight of the sample. This calculation provides an estimate of the organic carbon content of the sample in mg/g dry weight.
13. A cellulose standard is combusted with each batch of samples to insure that the sample combustion is complete and a laboratory replicate sample is analyzed at a frequency of one replicate every ten samples.

IV. TROUBLESHOOTING

Discussed in Methods sections.

V. STATISTICAL ANALYSIS AND DATA USAGE

Not applicable.

VI. REFERENCES

Dean, W.E., Jr., 1974. Determination of carbonate and organic matter in calcareous sediments and sedimentary rocks by loss-on-ignition: Comparison with other methods, *Journal of Sedimentary Petrology*, 44: 242-248.

POINT OF CONTACT:

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I. OBJECTIVE

Collection and identification of invertebrate animals from subtidal sediments for analysis of sediment dynamics, pollutant uptake pathways, and response of faunal assemblages to stress.

II. MATERIALS AND EQUIPMENT

Grab sampling operations will be combined with these for "subtidal sediment chemistry sampling" (see SOP, J.W. King, URI/GSO equipment list). Of the two samplers which will be available for this project, the teflon-coated Van Veen grab is preferred because of its extensive use in EPA shallow water monitoring. The following equipment for shipboard use not listed in the chemistry sampling SOP include:

- 12" dia. 0.5mm mesh stainless steel sieve
- stainless steel spoons and forceps
- waterproof labels (Nalge polypaper)
- 20 liter plastic basin
- 8 and 14 qt polypropylene buckets with lids
- 25cm dia., 15cm long clear plastic core tube
- soil color chart (Munsell)
- SLR camera and film
- plastic cm scale

The following equipment will be used in the laboratory:

- 12" dia. 0.5mm sieve
- 8" dia 2.0, 1.0, and 0.5mm sieves
- 20 liter plastic basin
- low velocity source of filtered seawater
- 1 liter glass jars
- stock solution of $MgCl_2$ (500g/l)
- stock solution of formalin with Rose Bengal stain and borax buffer
- brass wire brush
- 3 liter tall-form pitcher
- heavy glass sorting trays
- 90mm dia glass petri dishes
- fine stainless steel forceps
- low power binocular microscopes

- high power microscope
- 70% ethyl alcohol
- 0.9ml shell vials
- 60ml snap cap wide mouth vials

III. METHODS

- 1) Shipboard - Shipboard methods closely follow those of Reifsteck et al. (1993) used in the EMAP Program. (15.1: Sediment Collections, 15.1.1: Field processing of samples for benthic community assessment). They include the following steps:
 - a) rinse the grab with seawater from the station being sampled before use
 - b) add weight necessary for the sediment type and adjust if necessary
 - c) lower grab at a rate of less than 1m/sec. through the last 5 meters
 - d) accept grab samples if they are level, intact over entire area and with a depth at center of at least 7cm (EMAP Fig. 15.1) For this project shell-covered substrate is acceptable
 - e) drain seawater overlying the sampled sediment in a 20 liter plastic basin, sieve to 0.5mm and return organisms to sediment
 - f) photograph sediment surface with a cm scale and sample label, record color and surface features of sediment
 - g) measure depth of sediment at middle of sample
 - h) take 2.5cm dia. core and measure the depth and form of the apparent redox potential discontinuity (RPD)
 - i) empty grab into basin and basin into transport bucket. Wash grab and basin into 0.5mm sieve and place residue in bucket with sample lable. Place lid on bucket and store it in a cool location
- 2) Laboratory - Laboratory methods vary from those described in Klemm and others (1994) to fit the requirements of this study.
 - a) Sample preparation - Because of the nearness of the sample sites to the Graduate School of Oceanography it is possible to process samples in the laboratory under controlled conditions with no necessity to hurry to accommodate shipboard operations. The following procedures will be utilized.
 1. Suspend sediment in a low velocity flow of filtered seawater and allow to pass through a 12" dia. 0.5mm sieve. Place remaining sediments in the sieve within a seawater filled basin. Move sieve up and down until only small particles of cohesive sediment remain (allowing free access of relaxant and preservative to organism).

2. Remove residue and organisms with label to a jar containing an 8% solution of $MgCl_2$ and hold for approximately 30 minutes.
 3. Pour off overlying fluid through a 0.3mm sieve, return any particles to jar, and preserve with a stock formalin solution to obtain a 10% concentration. Label the outside of the jar (secondary to inner tag).
 4. Clean sieves with bronze brush and high water flow.
- b) Sorting - Benthic organisms alive at the time of collection are separated from coarse sediments, shells, and organic detritus. Sieving and elutriation in tap water are used to remove preservative, stain, and fine sediment and to separate the sample into fractions with relatively uniform grain size and density which increases speed and accuracy of sorting. Particles retained on a 2mm sieve are sorted without magnification. All finer fractions are sorted under binocular microscopes. Microfaunal taxa in which the majority of individuals are too small to be retained on a 0.5mm sieve will not be removed; these include nematodes, harpacticoid copepods, and mites. Pelagic calanoids, cladocerans, and crab zoea will also not be removed. Juvenile amphipods will be removed only if they are living independently of adults as evidence by food in the gut. Individuals of colonial epifaunal species such as tunicates and hydroids will not be counted.
- c) Identification and Enumeration - Commonly occurring species will be separated, counted, and preserved in 70% alcohol during initial sorting. Rare, difficult to identify, and damaged species will be preserved in major taxonomic groups for later examination and comparison with other samples. The majority of individuals will be sorted to the species level. For some groups such as nemertines, turbularians, leeches, and oligochaetes, it is not possible to identify species without special killing and preservation techniques or examination of internal organs under high power magnification. Where types within these groups can be recognized they will be assigned letter designations. To facilitate diversity analysis small or damaged specimens similar to identified specimens will be given that name and not reported as "unidentified."

The length of all bivalves will be recorded at notes made of the dominant size classes of the other taxa. All identified material will be preserved in 70% alcohol and archived for possible analysis of feeding type, condition, population structure, and growth rate. Counts will be entered on an Excel spreadsheet.

All taxonomic differences between this proposed survey and the (1985) Menzie-Cura & Associates (1994) survey will be resolved in order to make direct comparison possible. Major differences with Narragansett Bay EMAP sample data and City of Newport outfall study data will also be resolved.

V. STATISTICAL ANALYSIS AND DATA USAGE

Detailed statistical analysis is not included in these procedures, however the data will be described in ways which will allow the design of tests of significance of disposal site effects. The spatial distribution of densities of dominant species; indicator species; and community parameters such as number of species, density, evenness, and proportion of deposit feeders will be mapped. Correlation coefficients between species, and between species and sediment/contaminant variables will be noted. The index under development by the EMAP program to identify stressed benthic communities will be calculated for all stations. Variation in species density will be discussed in terms of major habitat gradients and small scale patchiness. Relatively homogeneous subareas will be identified for potential use in univariate and multivariate tests of significance.

VI. REFERENCES

- City of Newport. 1985. Application for modification of secondary treatment requirements for its water pollution control plant discharge into marine waters. Metcalf and Eddy, Inc., Boston, MA.
- Klemm, D.J. and others. 1994. Environmental Monitoring and Assessment Program (EMAP) Laboratory Methods Manual Estuaries. Vol. 1 - Biological and Physical Analyses. EPA/620/A-94/XXX. U.S. EPA Environmental Monitoring Systems Laboratory, Cincinnati, OH.
- Menzie-Cura & Associates, Inc. 1994. Assessment of marine Benthic Infauna and Epifauna in Vicinity of Naval Education and Training Center Sites. Prepared for TRC Environmental Corp.
- Reifsteck, D.R., C.J. Strobel, D. Keith. 1993. Environmental Monitoring and Assessment Program (EMAP) Near Coastal Component: 1993 Virginian Province Effort Field Operations and Safety Manual. EPA/600/X93/XXX. U.S. EPA Environmental Research Laboratory, Narragansett, RI.

POINT OF CONTACT:

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I. OBJECTIVE

This SOP describes the procedure for analysis by graphite furnace atomic absorption spectrophotometry.

II. NECESSARY MATERIALS AND EQUIPMENT

- Perkin-Elmer 4100ZL graphite furnace atomic absorption spectrophotometer

III. METHODS

Where conditions for a particular element and instrument are not specifically provided, the instrument manufacturer's recommended operating conditions and parameters are used.

A. Instrumental Setup

1. Turn power on computer , printer, GFAA, and Exhaust fan.
2. Double click on AA_INST.EXE Starts Benchtop Software.
3. Install appropriate Lamp in turret and align.
4. Fill DI rinse bottle.
5. Select operating conditions from menus in software.
 - a. Element
 - b. Element Parameters (use defaults)
 - c. Furnace (autodefine)
 - d. Calibration (set calibration standards , matrix modifiers, and samples)
 - e. QC (enter location and frequency of check samples)
6. Enter sample name and other sample information into Element Weight ID File

B. Calibration and sample Analysis

1. Standards should be chosen to bracket expected absorbance ranges.
2. A Blank and 4 standards are the minimum run.
3. A calibration run is made and if satisfactory samples are run.
4. All sample analysis is run with appropriate check samples and recalibration if a checksample does not meet with criteria set. All measurements use Peak area for calculations. All samples are measured twice and an average of the two measurements is used.

Analyses are performed using THGA Graphite tube with integrated L'vov-type platform, maximum power heating, zero-gas flow during atomization and Zeeman background correction. Peak areas used for calibration and quantitation. All analyses utilize 20 ul sample injections + matrix modifier dependent on the element.

Further information on individual elements can be found in URI/GSO Modification of ERL-N SOP 2.04.006.

IV. TROUBLESHOOTING

Discussed in methods section.

V. STATISTICAL ANALYSIS AND DATA USAGE

1. Calibration curves relating absorbance to solution concentration are generated by linear or polynomial regression of absorbance against concentration for the calibration standards. This is performed automatically by the program.
2. These curves are used to convert sample absorbances to concentrations.
3. Metals concentrations determined in the standard reference materials (SRMs), prepared with the samples as unknowns, should be compared with reference values and confidence intervals to confirm accurate preparation and analysis of the metals.

VI. REFERENCES

None.

POINT OF CONTACT:

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I. OBJECTIVE

This SOP describes the procedure for mercury analysis by mercury analyzer.

II. NECESSARY MATERIALS AND EQUIPMENT

- Leemen Labs Mercury Analyzer Model PS200

III. METHODS

The instrument manufacturer's recommended operating conditions and parameters are used.

A. Make up drying tubes of Magnesium Perchlorate

1. Clean glass drying tubes and dry.
2. Fill with Magnesium Perchlorate and plug ends with glass wool.
3. Place in desiccator.
4. Place new tube in system before starting.

B. Starting Instrument PS200

1. Turn on surge protector to start computer.
2. Turn on Argon Gas.
3. Press green button on lower right of PS200
4. Press blue button to turn on lamp.
5. Start PS200 program.
6. Press F2 Macro key.
 - a. Type COLDSTART and press Enter- Note coldstart procedure takes 2¹/₂ hours!

C. Prepare Standards of 5, 2, 1, and .5 ppb (ng/g).

1. .050g = 50 ul of 100ppm Hg standard. dilute to 5.00g with 10% HCL.
Actual concentration 1ppm = 1,000ppb
2. 100ppb standard dilute above 1:10
 - a. 1g of 1,000ppb diluted to 10g with 10% HCL or
 - b. 0.5g of 1,000ppb diluted to 5g with 10% HCL.
3. Dilute 100ppb standard to make 5, 2, 1, and .5 ppb (ng/g).
 - a. 5ppb
1.25g of 100ppb diluted to 25g.
 - b. 2ppb
.5g of 100ppb diluted to 25g.

c. 1ppb

.25g of 100ppb diluted to 25g.

d. .5ppb

.125g of 100ppb diluted to 25g.

D. Run Aperture test. 2.10 in manual.

E. Initial system test ~ 40 min.

1. Macro F2
2. Type Systest Enter

F. Running Samples 3.3 in manual.

Note. Whenever you copy or create a protocol, you must create a new folder. (the folder is where all data is stored).

Samples take ~ 2min. to run 88 samples takes about 2¹/₂ hours.

IV. TROUBLESHOOTING

Discussed in methods section.

V. STATISTICAL ANALYSIS AND DATA USAGE

1. Calibration curves relating absorbance to solution concentration are generated by linear or polynomial regression of absorbance against concentration for the calibration standards. This is performed automatically by the program.
2. These curves are used to convert sample absorbances to concentrations.
3. Metals concentrations determined in the standard reference materials (SRMs), prepared with the samples as unknowns, should be compared with reference values and confidence intervals to confirm accurate preparation and analysis of the metals.

VI. REFERENCES

None.

Point of Contact:
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1. OBJECTIVE

This document describes the procedures used to extract pore-water from estuarine and freshwater sediments using a vacuum-operated system following methods described in Winger and Lasier (1991). Actual volumes needed for analyses and biological testing and holding requirements are task-specific and require consultation with the Project Manager.

2. SAFETY

Sediments may be contaminated with hazardous biological or chemical constituents. Proper attire should be worn.

3. MATERIALS

Fused-glass air stone
Hose clamp
Medical grade silastic tubing (3/16" I.D.)
30 or 60 cc polypropylene syringe with catheter tip
Piece of wood cut to fit between the end of the syringe and the lip of the plunger
0.45 μ m filter unit
50-ml centrifuge tube
Deionized water in squirt bottle

4. METHODS

- 4.1 Label the syringes and 50-ml centrifuge tubes with data transcribed from the sample container.
- 4.2 Use tubing to attach fused-glass air stone to the syringe.
- 4.3 Insert air stone 8 to 10 cm into the sediment sample.

- 4.4 Secure the tubing to container with elastic bands.
- 4.5 Attach hose clamp to the tubing.
- 4.6 Create a vacuum by loosening the hose clamp retracting the plunger of the syringe.
- 4.7 Brace the plunger in the retracted position by inserting a piece of wood between the end of the syringe and the lip of the plunger.
- 4.8 Collect 2 to 5 ml of pore-water.
- 4.9 Maintain vacuum by tightening hose clamp.
- 4.10 Remove the brace.
- 4.11 Remove the syringe.
- 4.12 Discard pore-water.
- 4.13 Rinse syringe and plunger with deionized water from squirt bottle.
- 4.14 Reassemble and reattach syringe to tubing.
- 4.15 Reestablish the brace.
- 4.16 Loosen the hose clamp.
- 4.17 Fill the syringe. For fine-grained sediments, 12 - 18 hours may be required. Store sample in at 4°C in the dark during extraction.
- 4.18 Remove the airline tubing from the syringe when the syringe is full.
- 4.19 Examine pore-water for turbidity. Filter if necessary. Multiple collections require homogenization before filtration.
- 4.20 Dispense the pore-water from the syringe or the filter unit into a pre-labeled 50-ml centrifuge tube. Multiple collections require homogenization before dispensation into 50-ml tubes.

4.21 Store sample at 4°C in the dark for further analyses.

4.22 Sediments are discarded in waste barrels for disposal. Barrels are sampled and analyzed to establish proper disposal procedures. Disposal procedures are performed in accordance with local, state, and federal disposal regulations.

5.0 QA/QC

Any deviations from this SOP are documented and reported to the Program Manager.

6.0 REFERENCES

Winger, PV and PJ Lasier. 1991. A Vacuum-Operated Pore-Water Extractor for Estuarine and Freshwater Sediments. Arch. Environ. Contam. Toxicol. 21, 321-324.

Point of Contact:
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1. OBJECTIVE

This document describes the procedures used to prepare cytological blood smears for diagnosis of hematopoietic neoplasia in the bivalve molluscs following methods described in Morrison *et al.* (1993).

2. SAFETY

Clams may be contaminated with hazardous biological or chemical constituents. Proper attire should be worn. Staining procedures require the use of carcinogenic and explosive chemicals. These procedures are performed under portable fume hoods. Chemicals are stored under appropriate and secure conditions.

3. MATERIALS

Coolers
Blue ice
Absorbent towels
Alcohol
Permanent marker
Aquaria for holding, with water bath and pump and tubing for air or flow-through system
Plastic 1 ml disposable syringes with 26 gauge needle
Chilled (5°C) filtered (0.45 µm) seawater (15 ppt)
Filter unit with 0.45 µm filter
1 Coplin jar filled with fixative
 1% glutaraldehyde
 4% formaldehyde
 42.5% filtered (0.45 µm) seawater
 42.5% DI
3 Coplin jars filled with tap water

- 5 beakers filled with DI for dipping
- 1 beaker filled with 1:9 ethanol/butanol for dipping
- 1 Coplin jar filled with 5 N HCl
 - 583.5 ml DI
 - 416.5 ml concentrated HCl
- 1 Coplin jar filled with Schiff reagent
 - Boil 200 ml DI
 - Add 1 g basic fuchsin immediately
 - Cool to 60°C
 - Add 20 ml 1 N HCl
 - 916.5 ml DI
 - 83.5 ml HCl
 - Cool to 25°C
 - Add 1.5 g sodium metabisulfite ($\text{Na}_2\text{S}_2\text{O}_5$)
 - Stopper and store in dark at room temperature for 18 to 24 hr
 - Add .5 g activated charcoal
 - Shake vigorously for one minute
 - Filter (0.45 μm)
 - Store clear to light yellow solution tightly capped in amber bottle in dark at 0 - 5°C
- 1 Coplin jar filled with picromethyl stain
 - 500 ml DI
 - .15 gm methyl blue crystals
 - 5.9 g picric acid crystals
- 2 Coplin jars filled with butanol
- 2 Coplin jars filled with xylene
- 8 chambered tissue culture slides
- Coverslips
- Synthetic mounting media
- Compound microscope with 40 and 100X objectives
- Oil immersion

4. METHODS

4.1 Transport and Holding

4.1.1 Set up aquaria for holding containing ambient seawater with aeration maintained in water bath or use flow-through ambient system.

4.1.2 Transport clams in insulated coolers with blue ice.

4.1.3 Upon arrival, place clams in flow-through ambient system for holding.

4.2 Labeling

4.2.1 For processing, dry clams with absorbent towels, clean with alcohol, air dry, and label with permanent, indelible marker.

4.2.2 Label each clam with lab ID# by station and by replicate with a unique identifier (e.g., Station 1/Replicate A3/Lab ID# 1, Station 1/Replicate A3/Lab ID #2, Station 1/Replicate A3/Lab ID# 3.....).

4.3 Slide Preparation

4.3.1 Use plastic 1-ml disposable syringe fitted with 26-gauge needle filled with 0.9 ml chilled (5°C) filtered (0.45 µm) seawater at 15 ppt to draw hemolymph from the pericardial region or the adductor muscle (see Figure 1).

4.3.2 Insert needle between the valves into either the anterior or posterior muscle (10 - 15 mm penetration).

4.3.3 Aspirate 0.1 ml of hemolymph into the syringe.

4.3.4 Remove the needle and dispense .5ml of diluted hemolymph into each upper (A1, B1, C1, D1) and lower (A2, B2, C2, D2) slide chambers per animal (see Figure 2).

4.3.5 Allow cells to settle for one hour.

4.3.6 Label the slide. Slides for each project are numbered consecutively (e.g. 1, 2, 3.....). Data sheets are used to record the slide number, chamber, station, replicate, lab ID#, and the date processed (see Attachment A).

4.3.7 Return clam to holding aquaria until slides have been examined for overall quality.

4.3.8 Remove slide superstructure and gasket.

4.4 Fixing and Hydrolyzing

- 4.4.1 Place slide in fixative for 10 min. Do not allow slide to air dry between treatments.
- 4.4.2 Place slide in tap water for 5 min. Do not allow slide to air dry between treatments.
- 4.4.3 Dip slide in first DI rinse 6 times. Do not allow slide to air dry between treatments.
- 4.4.4 Hydrolyze slide in 5 N HCl for 30 min. Do not allow slide to air dry between treatments.
- 4.4.5 Place slide in tap water for 2 min. Do not allow slide to air dry between treatments.
- 4.4.6 Dip slide in second DI rinse 6 times. Do not allow slide to air dry between treatments.
- 4.4.7 Dip slide in third DI rinse 6 times. Do not allow slide to air dry between treatments.

4.5 Staining

- 4.5.1 Place slide in Schiff reagent (5°C) for 15 min. Do not allow slide to air dry between treatments.
- 4.5.2 Place slide in tap water for 5 min. Do not allow slide to air dry between treatments.
- 4.5.3 Dip slide in fourth DI rinse 6 times. Do not allow slide to air dry between treatments.
- 4.5.4 Dip slide in fifth DI rinse 6 times. Do not allow slide to air dry between treatments.
- 4.5.5 Place slide in picromethyl stain for 3 min. Do not allow slide to air dry between treatments.
- 4.5.6 Dip slide in 1:9 ethanol/butanol 6 times. Do not allow slide to air dry between treatments.

4.5.7 Place slide in first butanol solution for 2 min. Do not allow slide to air dry between treatments.

4.5.8 Place slide in second butanol solution for 3 min. Do not allow slide to air dry between treatments.

4.6 Mounting

4.6.1 Place slide in first xylene solution for 5 min. Do not allow slide to air dry between treatments.

4.6.2 Place slide in second xylene solution for 5 min. Do not allow slide to air dry between treatments.

4.6.3 Mount with coverslip and synthetic mounting media.

4.7 Examination

4.7.1 Examine slide microscopically. Nuclei and DNA are red; cytoplasmic matter is blue.

4.7.2 Count 1000 cells. Normal cells are elongated with pseudopodia. Neoplastic cells are rounded up with no pseudopodia and large nuclei.

4.7.3 Data sheets are used to record normal, abnormal, and neoplastic cells (see Attachment B).

4.8 Archival

Slides are stored indefinitely in the dark at room temperature.

5.0 QA/QC

Any deviations from this SOP are documented and reported to the Program Manager.

6.0 References

Morrison, CM, AR Moore, VM Marryatt and DJ Scarratt. 1993. Disseminated

sarcomas of soft-shell clams, *Mya arenaria* Linnaeus 1758, from sites in Nova Scotia and New Brusnwick. Journal of Shellfish Research. Volume 12, No. 1, 65-69.

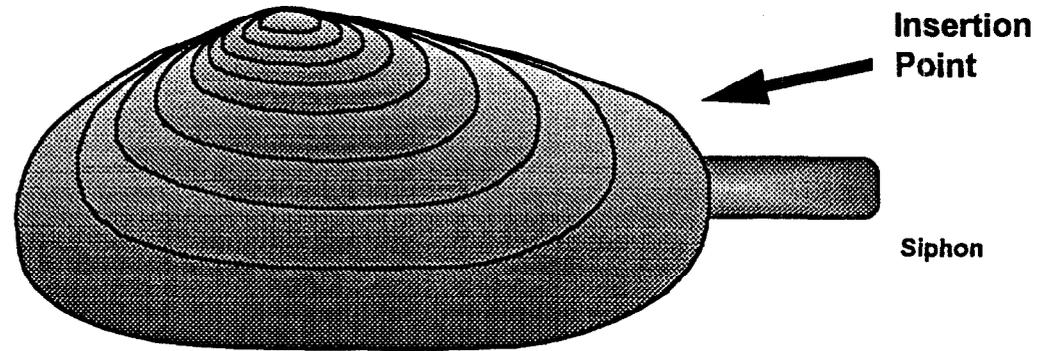


Figure 1. *Mya arenaria* showing location of syringe insertion.

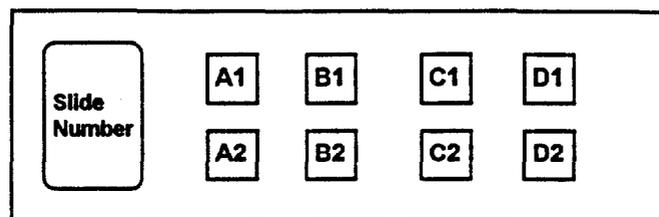


Figure 2. Illustration of slide and slide chambers used for histocytological preparation of hemolymph cells.

ATTACHMENT A

ATTACHMENT B

Point of Contact:
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1 OBJECTIVE

This document describes the methods used to conduct biota sampling of bivalves and fish. The purpose of the collection is to obtain indigenous bivalves and mummichog fish samples for assessment of chemical exposure and histological abnormalities for input into ecological risk assessments. A sample is one field duplicate consisting of a single species collected at a single field station. Four duplicate field sampling events are attempted at each station.

2. SAFETY

Environmental samples may contain hazardous biological or chemical constituents. Proper attire should be worn. Personnel required to sample in proximity of hazardous waste sites must have completed 40 hr OSHA training and yearly 8 hr refreshers.

3. MATERIALS

The materials and equipment required for biota sampling will vary depending upon the location of sampling, the species to be collected and the type of vessel used for sampling. The Principal Investigator should determine the best sampling approach prior to the sampling event.

Cooler with blue ice
1-gal Plastic bags
Aluminum foil
Water proof markers
Water-proof field notebook
Chain of Custody forms
Quadrant location sheets
Shovels, rakes
Canoe with Safety Gear, paddles
Cell Phone

Differential GPS receiver
4 x 4 meter quadrant locator
2 1-m² quadrants
Waders, gloves
Scrub brush
Sieve
Fish traps, stakes and line
Smith MacIntyre grab sampler, 0.1 m²,
Smith Mac stand, weights, cocking bar
Van Veen Grab Sampler

Hard hats
Face Shield

Respirator -if conditions dictate
Standard safety equipment (i.e. first-aid kit).
Sample containers
Exposure suits and/or foul weather gear including steel-toed boots

4. NEARSHORE/INTERTIDAL SAMPLING METHODS

4.1 Fish Sampling

- 4.1.1. Consult Work Plan for station locations. Survey sampling stake in with GPS navigation. Locate stake in 2' of water at low tide. Attach fish trap to stake with line. Add bread to cage as bait.
- 4.1.2. Check traps at daily intervals.
- 4.1.3. Retrieve fish traps, put fish in pre-cleaned glass jars, sorted by species. label as follows:
 - Study: MCA-NSB (for example)
 - Station: W-8 (for example)
 - Date: 11/20/94 (example collection Date)
 - Time: 13:54 (example collection end time)
 - Species: Mummichogs (for example)
- 4.1.4. Log all duplicate samples on Chain-of-custody sheets.

4.2 Locate Bivalve Sample Stations

- 4.2.1. Consult Work Plan for station locations and direction as to whether quantitative bivalve sampling is required and for station locations.
- 4.2.2. Locate survey stake in center of station and obtain position DGPS navigation to $\pm 2-5$ m accuracy using Trimble Pathfinder Basic + GPS unit or equivalent.
- 4.2.3. For non-quantitative sampling, 1 field duplicate is sampled and consists of an area within a 5 m radius of the navigated station position.
- 4.2.4. For quantitative sampling, locate 4x4-m² quadrant frame at station with stake at center position.
- 4.2.5. Use randomized block design, Select 4 replicates of 16 through random number table, preferably in advance of field travel. Mark results on sample sheets for field reference according to row and column position on sample sheet.

4.3 Sample Biota

4.3.1 Sample Epifauna: (Blue Mussels, Oysters)

Hand Pick, wear gloves. Obtain 25 animals greater than 1 cm, or as many as possible for each station duplicate as required.

4.3.2 Sample Shallow Infauna (Ribbed Mussels, Quahogs)

Rake Surface to 6" depth. Obtain 25 animals greater than 1 cm, or as many as possible.

4.3.3 Sample Deep Infauna (Soft Shell Clams)

Shovel out to 16" depth. Examine each shovel-full with care not to break shells. Obtain 25 animals greater than 1 cm, or as many as possible.

4.3.4 Clean excess sediment off all animals with brush.

4.3.5 Put duplicate samples into Ziploc plastic bags, Mark bag with:

Study: MCA-NSB (for example)
Station: W-8 (for example)
Duplicate: D-4 (for example)
Species: Mercenaria (for example)
Date: 11/20/94 (example collection Date)
Time: 13:54 (example collection end time)

4.3.6. Aggregate duplicate samples by station. Combine duplicates into a common "station" bag. Put soft shell species samples on top.

Mark bag with:

Study: MCA-NSB (for example)
Station: W-8 (for example)
Date: 11/20/94 (example collection Date)

4.3.7 Put bags in coolers with blue ice, not in direct contact with ice

4.3.8 Complete chain of custody sheet, logging all duplicates. Record Study, Date, Station, Sample Time and collectors.

4.3.9 Proceed to Step 6: Laboratory Processing Methods

5. OFFSHORE SAMPLING METHODS

5.1 Lobster Sampling

5.1.1. Consult Work Plan for station locations and depths.

5.1.2. Prepare lobster trap arrays with trap, line and float. Only coated-wire cages are permissible. Consult station depths to determine proper scope on line. Label floats with station number and shellfish collection permit number per RIDEM regulations. Bait trap with fish heads.

5.1.3. Notify DEM if sampling in areas closed to shellfishing.

5.1.4. Deploy traps at desired station locations. Note GPS position at time of

trap drop. (Do not rely on float position).

- 5.1.5. Check traps at two-day intervals. Retrieve all lobster from traps, wrap animals in seawater moist cheese cloth. Put wrapped specimens into ziploc plastic bags.

Label as follows:

Study: MCA-NSB (for example)
Station: W-8 (for example)
Date: 11/20/94 (example collection Date)
Time: 13:54 (example collection end time)
Species: Mummichogs (for example)

- 5.1.6. Complete chain of custody sheet, logging all duplicates. Record Study, Date, Station, Species, Sample Time and collector.
- 5.1.7. Proceed to Step 6: Laboratory Processing Methods.

5.2. Bivalve sampling

- 5.2.1. Consult Work Plan for station locations and depths.

- 5.2.2. Prepare Smith MacIntyre grab, Van Veen Grab, clam rake or bivalve dredge as appropriate. Consult station depths and substrate type to determine proper equipment.

- 5.2.3. Notify DEM if sampling in areas closed to shellfishing.

- 5.2.4. Locate desired stations using DGPS positioning. (Do not rely on LORAN or GPS position).

- 5.2.5. Using selected sampling equipment, obtain 25 animals of each species of a size greater than 1 cm, or as many as possible.

- 5.2.4 Clean excess sediment off all animals with brush.

- 5.2.5 Put duplicate samples into Ziploc plastic bag, Mark bag with:

Study: MCA-NSB (for example)
Station: W-8 (for example)
Duplicate: D-4 (for example)
Species: Mercenaria (for example)
Date: 11/20/94 (example collection Date)
Time: 13:54 (example collection end time)

- 5.2.6. Aggregate duplicate samples by station. Combine duplicates into a common "station" bag. Put soft shell species samples on top.

Mark bag with:

Study: MCA-NSB (for example)
Station: W-8 (for example)
Date: 11/20/94 (example collection Date)

- 5.2.7 Put bags in coolers with blue ice, not in direct contact with ice
- 5.2.8 Complete chain of custody sheet, logging all duplicates. Record Study, Date, Station, Species, number of duplicates, Sample Time and collectors.
- 5.2.9. Proceed to Step 6: Laboratory Processing Methods.

6. LABORATORY PROCESSING METHODS

6.1. Bivalve depuration

- 6.1.1 Select bivalve samples to be depurated in consultation with Principal Investigator. For all remaining samples, proceed to step 6.2, below.
- 6.1.2 Transport appropriate samples to ARC for depuration.
- 6.1.2 place in field duplicates in separate baskets with labeling
- 6.1.3 place baskets in round tank, 24-30 hr
- 6.1.5 Sub-sample 5 Mya/rep to remain in tank for subsequent testing
- 6.1.4 retrieve all other samples, rewrap in foil by duplicate, place in station bags and coolers with blue ice.
- 6.1.5. Transport cooler(s) to 4°C ETC refrigerator for temporary storage.
- 6.1.6 Complete chain of custody sheet, logging all reps note Study, Date, Station, Rep, Start Depuration Time, and depurator(s), cooler temperature upon arrival.
- 6.1.7. Log samples on refrigerator log, verify holding temperature.
- 6.1.8. Proceed to Step 6.2: Sample archival procedures.

6.2 Sample Holding Procedures

- 6.2.1 Return with cooler to ETC. Unpack samples and place in "project" box.
- 6.2.2 Verify samples collected agree with chain of custody sheet, through comparison with Study, Date, Station information on sample bags.
- 6.2.3. Certain analytical laboratories prefer whole animals while others prefer tissue only samples. For those laboratories requesting whole animals, a random selection of the required number of individuals can be made at this time.
- 6.2.4. These samples should be packaged as done previously and transported on dry ice to the requesting laboratory with chain of custody sheets.
- 6.2.3. Return samples to project box in -20°C freezer, Note change in logged samples on freezer log. And document removal of sample material and removal date on Chain of Custody form.
- 6.2.4. For laboratories requesting tissue only material, proceed to step 6.3.

6.3. Sample Extraction Procedures

- 6.3.1. Arrange for use of "clean lab" for all subsequent steps.
- 6.3.2. Assemble appropriate sample dissection equipment including cleaned glass jars with liners (supplied by analytical chemistry labs), titanium shucking knives, calipers, logbooks and data sheets.
- 6.3.3. Select only 1 set of station samples at a time from out of cold storage "project" box. Keep in cooler at lab bench.
- 6.3.4. Retrieve 1 duplicate sample from cooler. Transcribe label information to data sheet. Note also current date, time and personnel involved.
- 6.3.5. Remove foil package from bag and set bag aside. Unwrap foil over clean countertop.
- 6.3.6. Measure each of 25 ind/species/rep to nearest 0.1 mm. Record data on approved data sheets (1 rep/sheet), note total number of animals for each field duplicate found in sample.
- 6.3.7. Randomly divide available number of specimens among the required sample types (e.g. Metals, PCB/PAH/Pest, Butyltins, Condition).
- 6.3.8. Using the titanium knife, shuck tissues from all organisms for each sample type into the appropriate glass container with proper labeling. The jar should be kept on dry ice to prevent rethawing of the sample.
- 6.3.9. Repeat procedures 6.3.4-6.3.8 for remaining field duplicates of the same sample type. With the exception of condition, tissue material may be composited into the same sample type jar, unless field duplicate analyses are called for. Verify this information with the Principal Investigator.
- 6.3.10. Condition material is processed by duplicate for to determine sample dry weight. Samples are placed in pre-tared, covered weighing pans or beakers and placed in drying oven at 60-80°C for 24 hr. Samples are recovered and weighed to nearest 0.1 g.
- 6.3.10. Samples jar to be sent out should be sealed tightly and labeled and packaged appropriately to prevent breakage. Sample cooler should be clearly labeled as "Fragile" "Perishable Material" and "DRY ICE" according to DOT procedures as well as name and address of receiving laboratory. Chain of custody sheets are placed in ziploc bags in the cooler. The cooler is then shipped Federal Express Overnight Delivery. A call is made to alert the receiving lab of sample shipping and again to confirm sample receipt. Copies of chain of custody sheets are faxed back to ETC.

7.0 QA/QC

Note any deviations in sample plan in writing, inform program manager.

MOST PROBABLE NUMBER METHOD FOR THE ENUMERATION OF TOTAL COLIFORMS AND FECAL COLIFORMS IN SHELLFISH

Point of Contact

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The term "total coliform" refers to a group of gram-negative aerobic to facultative anaerobic, non-spore forming, rod shaped bacteria which ferment lactose at $35.0 \pm 0.5^\circ\text{C}$ in 24-48 hours. Included in this group are the genera *Escherichia*, *Enterobacter*, *Citrobacter* and *Klebsiella*. These organisms are widely distributed in nature and many are native to the gut of warm blooded animals, including man. The term "fecal coliform" refers to the thermotolerant forms of the total coliform group which ferment lactose at $44.5 \pm 0.2^\circ$ in 24 hours. Within this group, *Escherichia coli* and *Klebsiella sp.* are of interest since, when present, they indicate that recent fecal contamination has occurred. Enumeration of total coliforms and fecal coliforms using the most probable number method is a means of determining the sanitary quality of shellfish meats.

1.0 Necessary Materials and Equipment

1.1 Glassware, Plasticware, Equipment and Disposable Supplies

- 2 liter Erlenmeyer flasks
- 6 x 50 mm culture tubes
- 16 x 100 mm test tubes
- 16 x 150 mm test tubes
- 16 and test tube caps and racks
- 1.0 and 10.0 ml sterile pipettes
- 20 liter polypropylene carboy
- Heating mantle with magnetic stirrer and stir bars
- Balance and weigh boats
- Autoclave
- Cornwall syringe
- Air incubators $35.0 \pm 0.5^\circ\text{C}$
- Waterbaths $44.5 \pm 0.2^\circ\text{C}$
- Sterile shucking knives and scrub brushes
- Waring blenders
- Timer

1.0 Necessary Materials and Equipment (continued)

1.1 Glassware, Plasticware, Equipment and Disposable Supplies

pH meter
Sterile transfer sticks

1.2 Media and Diluent

Lauryl Tryptose Broth
Brilliant Green Bile 2%
EC Medium
Potassium phosphate
Sodium hydroxide
Deionized water

2.0 Media and Diluent Preparation

2.1 Enrichment Media Preparation: Single Strength Lauryl Tryptose Broth

- 2.1.1 Weigh 35.6 g of lauryl tryptose broth into a 2 liter Erlenmeyer flask.
2.1.2 Add 1.0 liter of deionized water and bring to a boil with continuous stirring.
2.1.3 Dispense the medium in 10.0 ml aliquots into 16 x 150 mm test tubes containing inverted 6 x 50 mm culture tubes.
2.1.4 Cap the test tubes and autoclave at 121°C for 15 minutes, total time of heat exposure **MUST** not exceed 45 minutes.
2.1.5 Store the media at 4°C in the dark for up to one month.

2.2 Total Coliform Confirmation Media Preparation: Brilliant Green Bile 2%

- 2.2.1 Weigh 40.0 g of Brilliant Green Bile 2% into a 2 liter Erlenmeyer flask.
2.2.2 Add 1.0 liter of deionized water and warm slightly with continuous stirring to completely dissolve the medium.
2.2.3 Dispense the medium in 5.0 ml aliquots into 16 x 100 mm test tubes containing inverted 6 x 50 mm culture tubes.
2.2.4 Cap the test tubes and autoclave at 121°C for 15 minutes, total time of heat exposure **MUST** not exceed 45 minutes.
2.2.5 Store media at 4°C in the dark for up to one month.

2.3 Fecal Coliform Confirmation Media Preparation: EC Medium

- 2.3.1 Weigh 37.0 g of EC medium into a 2 liter Erlenmeyer flask.
2.3.2 Add 1.0 liter of deionized water and bring to a boil with continuous stirring.
2.3.3 Dispense the medium in 5.0 ml aliquots into 16 x 100 mm test tubes containing inverted 6 x 50 mm culture tubes.
2.3.4 Cap the test tubes and autoclave at 121°C for 15 minutes, total time of heat exposure **MUST** not exceed 45 minutes.
2.3.5 Store media at 4°C in the dark for up to one month.

2.0 Media and Diluent Preparation (continued)

2.4 Stock Phosphate Buffer Solution: Buffered Dilution Water

- 2.4.1 In a 1 liter volumetric flask, dissolve 34.0 g of potassium phosphate in 500 ml of deionized water.
- 2.4.2 Adjust to pH 7.2 with 10N sodium hydroxide.
- 2.4.3 Make up to 1 liter with deionized water.
- 2.4.4 Add the buffer solution to a 1 liter polypropylene bottle and autoclave at 121°C for 15 minutes.
- 2.4.5 Store at 4°C in the dark for up to one month.

2.5 Diluent Preparation: Phosphate Buffered Dilution Water

- 2.5.1 Add 20 liters of deionized water to an autoclavable carboy.
- 2.5.2 Add 25 ml of stock phosphate buffer solution and mix thoroughly.
- 2.5.3 Autoclave at 121°C for 45 minutes.
- 2.5.4 Store at room temperature for up to two weeks.

2.6 Diluent Preparation: Phosphate Buffered Dilution Blanks

- 2.6.1 Add 1.0 liter of deionized water to a 2 liter Erlenmeyer flask.
- 2.6.2 Add 1.25 ml of stock phosphate buffer solution and mix thoroughly.
- 2.6.3 Dispense the medium in 9.2 ml aliquots into 16 x 150 mm test tubes, 81.0 ml aliquots into 100 ml graduated dilution bottles and 100.0 ml aliquots into 100 ml graduated dilution bottles.
- 2.6.4 Cap the test tubes and/or dilution bottles and autoclave at 121°C for 15 minutes.
- 2.6.5 Refrigerate at 4°C in the dark for up to one month. **NOTE: Always check the volume of dilution water present in dilution blanks with each use. Discard any batch of buffered dilution water where evaporation has resulted in an inappropriate volume of buffered dilution water.**

3.0 Sample Storage

3.1 Shellfish Storage Containers

- 3.1.1 Use containers for shell-stock which are waterproof and durable enough to withstand the cutting action of the shellfish. Waterproof paper bags and plastic bags are suitable containers.
- 3.1.2 Use sterile wide-mouth jars with watertight closure for freshly shucked shellfish samples. Transfer samples of the final product to the sample jar with sterile forceps or spoon.
- 3.1.3 Use one or two packages of frozen shucked shellfish, containing 10 to 12 animals each, as one sample. Transfer core samples taken from larger blocks to sterile wide-mouth jars for transportation to the laboratory.

3.0 Sample Storage (continued)

3.2 Shellfish Storage Conditions and Temperature

- 3.2.1 Keep shell-stock samples in dry storage at a temperature above freezing but lower than 10°C until examination. Do not allow samples to come in contact with ice.
- 3.2.2 Refrigerate samples of shucked shellfish immediately after collection by packing in crushed ice until examination.
- 3.2.3 Keep samples of frozen shucked shellfish in the frozen state or at temperatures close to those at which the commercial stock was maintained. When this is not possible, pack samples of frozen shucked shellfish in crushed ice until examination.

3.3 Shellfish Identification Records

- 3.3.1 Maintain records of identification for each shellfish sample to enter the laboratory. Include the date, time, and place of collection, area the shellfish were harvested, the date and time of harvesting and the physical and chemical measurements of the harvesting water (if applicable). Also include the storage conditions between harvesting and collection.
- 3.3.2 Mark individual containers of shellfish samples for identification. Also, include identification information on a descriptive form to accompany the sample.
- 3.3.3 Record the time elapsed between collection and examination for each shellfish sample examined in the laboratory. Examine samples of shell stock and shucked, unfrozen shellfish within 6 hours after collection and in no case examine shellfish if they have been held more than 24 hours after collection.
- 3.3.4 Record the identification of the shipper, the date of shipment, and the harvesting area, the date, time and place of collection for market shellfish samples when other specified information is not available.

4.0 Sample Preparation

4.1 Shellfish in the Shell - Cleaning the Shells

- 4.1.1 Use a minimum of 12 shellfish in order to obtain a representative sample (approximately 200 g of shellfish liquor and meats) and to allow for the selection of sound animals suitable for shucking. Collect at least 200 g of sample when examining shucked or frozen shellfish.
- 4.1.2 Scrub hands thoroughly with soap and water.
- 4.1.3 Scrape off all growth and loose material from the shell. Scrub the shell stock with a sterile stiff brush under running water of drinking-water quality, paying particular attention to the crevices of the shells.

4.0 Sample Preparation (continued)

4.1 Shellfish in the Shell - Cleaning the Shells

4.1.4 Place the cleaned shell stock in clean containers or on clean towels to drain in the air.

4.2 Removal of Shell Contents

4.2.1 Before starting the removal of shell contents, scrub hands thoroughly with soap and water and rinse with 70% alcohol.

4.2.2 **OYSTERS:** Hold the oyster in the hand or on a fresh clean paper towel on the bench with the deep shell on the bottom. Using a sterile oyster knife, insert the point between the shells on the ventral side (at the right when the hinge is pointed away from the examiner), about 1/4 the distance from the hinge to the bill. Entry may also be made at the bill. Cut the adductor muscle from the upper flat shell and pry the shell wide enough to drain the shell liquor into a sterile tared blender. The upper shell may then be pried loose at the hinge, discarded, and the meats transferred to a sterile tared blender after severing the muscle attachment to the lower shell.

4.2.3 **HARD CLAMS:** Enter the hard clam with a sterile, thin-bladed knife similar to a paring knife. To open the clam, hold it in the hand, place the edge of the knife at the junction of the bills, and force it between the shells with a squeezing motion. An alternative method is to nibble a small hole in the bill, and with the knife, sever the two adductor muscles. Drain the shell liquor into a sterile tared blender. Cut the adductor muscles from the shells and transfer the body of the animal to the blender.

4.2.4 **OTHER CLAMS:** Shuck soft clams, butter clams, surf clams and similar species with a sterile paring knife, entering at the siphon end and cutting the adductor muscles first from the top valve and then from the bottom valve. Drain the shell liquor into a sterile tared blender. Transfer the body of the animal to the blender.

4.2.5 **MUSSELS:** Remove the byssal threads during the cleansing of the shell. Enter the mussel at the byssal opening. Insert the knife and spread the shells apart with a twisting motion, draining the liquor of the shellfish into a sterile tared blender. Transfer the body of the animal to the blender.

4.3 Dilution and Grinding

4.3.1 Weigh the shellfish meats and liquors.

4.3.2 Add an equal amount, by weight, of phosphate buffered dilution water to the blender.

4.0 Sample Preparation (continued)

- 4.3.3 Grind for 60-120 sec in a sterile waring blender at approximately 14,000 RPM. Avoid excessive grinding to prevent overheating.

5.0 Sample Analyses

5.1 Presumptive Test Using Lauryl Tryptose Broth

- 5.1.1 Inoculate each of 5 single strength lauryl tryptose broth tubes with 2.0 ml of the prepared sample (equal to 1.0 g of shellfish), 5 single strength lauryl tryptose broth tubes with 1.0 ml of a 10 fold dilution of the prepared sample (equal to 0.1 g of shellfish) and 5 single strength lauryl tryptose broth tubes with 1.0 ml of a 100 fold dilution of the prepared sample (equal to 0.01 g of shellfish).
- 5.1.2 In order to avoid indeterminate results, perform extensions of the dilutions mentioned.
- 5.1.3 Incubate tubes at $35.0 \pm 0.5^\circ\text{C}$ for 24 ± 2 hours.
- 5.1.4 Examine tubes for the presence/absence of gas production.
- 5.1.5 Score tubes with gas production and perform confirmation tests at this time.
- 5.1.6 Return tubes to $35.0 \pm 0.5^\circ\text{C}$.
- 5.1.7 Re-examine the tubes at 48 ± 3 hours for the presence/absence of gas production.
- 5.1.8 Score tubes with gas production and perform confirmation tests at this time.
- 5.1.9 Absence of gas production at the end of 48 ± 3 hours constitutes a negative test result for the total and fecal coliform groups.

5.2 Confirmed Tests Using Brilliant Green Bile 2% and EC Medium

- 5.2.1 General Rules for Performing the Confirmed Test (when 3 or more replicate portions of a series of 3 or more decimal dilutions of sample are planted): Select the tubes of the highest dilution (smallest volume) in which all tubes show gas production in 24 hours. Submit all of these tubes as well as every one of the gas-positive tubes in all higher dilutions to the confirmed tests. If all tubes show gas production, submit all tubes of the highest dilution and of the next-to-the-highest dilution to the confirmed tests. Submit all tubes of all dilutions in which gas is produced only at the end of 48 hours incubation to the confirmed test. All tubes producing gas that have not been submitted to the confirmed test shall be recorded as containing organisms of the coliform group, even though all the confirmed tests may yield negative results.

5.0 Sample Analyses (continued)

5.2 Confirmed Tests Using Brilliant Green Bile 2% and EC Medium

- 5.2.2 Mix the presumptive lauryl tryptose broth tubes by gentle shaking or rotating.
- 5.2.3 Transfer sample using sterile transfer sticks from lauryl tryptose broth tubes to Brilliant Green Bile 2% and EC Medium tubes.
- 5.2.4 Incubate Brilliant Green Bile tubes at $35.0 \pm 0.5^\circ\text{C}$ for 48 ± 3 hours and EC Medium tubes at $44.5 \pm 0.2^\circ\text{C}$ for 48 ± 3 hours.
- 5.2.5 Examine tubes for the presence/absence of gas production.
- 5.2.6 Score Brilliant Green Bile 2% tubes with gas production positive for the total coliform group and EC Medium tubes with gas production positive for the fecal coliform group.
- 5.2.7 Score Brilliant Green Bile 2% tubes and EC Medium tubes with no gas production negative for the total and fecal coliform groups.

6.0 Using the Most Probable Number Geometric Series

- 6.1 Count the number of positive tubes from each of the three dilutions performed.
- 6.2 Use the number of positive tubes from each dilution to form a 3 digit code.
- 6.3 Locate your three digit code on the most probable number geometric series chart.
- 6.4 Read the MPN number which corresponds to your three digit code. This is your density per 100 g of sample.
- 6.5 If, instead of 10, 1.0, and 0.1 ml a combination of portions of 100, 10, and 1 ml is used, record the MPN as 0.1 times the figure in the tabulation. On the other hand, should a combination of corresponding portions of 1.0, 0.1 and 0.01 ml be planted, record 10 times the figure in the tabulation; should a combination of portions of 0.1, 0.01, and 0.001 ml be planted, record 100 times the figure in the tabulation.
- 6.6 When more than 3 dilutions are employed in a decimal series of dilutions, the results from only 3 of them are significant. To select the 3 dilutions to be used in determining the MPN index, using the system of 5 tubes of each dilutions as an example, choose the highest dilution which gives positive results in all of the 5 portions tested (no lower dilution giving any negative results) and the 2 next succeeding high dilutions. The results of these 3 volumes should then be used in the computations of the MPN index.

6.0 Using the Most Probable Number Geometric Series (continued)

In examples given below, significant dilutions results are shown in boldface. The number in the numerator represents positive tubes; that in the denominator, the total tubes planted.

	1 ml or g	0.1 ml or g	0.01 ml or g	0.001 ml or g
(a)	5/5	5/5	2/5	0/5
(b)	5/5	4/5	2/5	0/5
(c)	0/5	1/5	0/5	0/5
(d)	5/5	3/5	1/5	1/5
(e)	5/5	3/5	2/5	0/5

In example (c) above, the first 3 dilutions are taken in order to throw the positive result into the middle dilution. When a case arises such as is shown in (d), where a positive occurs in a dilution higher than the 3 chosen according to the rule, it is included in the result of the highest chosen dilution (e).

7.0 Trouble Shooting

- 7.1 Thoroughly clean the shells of each shellfish, especially the hinge regions.
- 7.2 Adequately mix samples and dilutions of samples prior to addition to enrichment tubes.
- 7.3 Adequately mix presumptive enrichment tubes prior to addition of sample to confirmation enrichment tubes.

8.0 Statistical Analysis and Data Usage

- 8.1 Tabulate and summarize the data.
- 8.2 Use data in conjunction with other indicator tests to determine sanitary quality.

9.0 References

- 9.1 American Public Health Association, Inc. 1970. Recommended Procedures for the Examination of Sea Water and Shellfish. Washington, DC. p. 1-105.
- 9.2 American Public Health Association, American Water Works Association, Water Pollution Control Federation. 1989. Standard Methods for the Examination of Water and Wastewater, Seventeenth Edition. Washington, DC. Chapter 9.

MOST PROBABLE NUMBER METHOD FOR THE ENUMERATION OF TOTAL COLIFORMS AND FECAL COLIFORMS IN SEDIMENT

Point of Contact

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The term "total coliform" refers to a group of gram-negative aerobic to facultative anaerobic, non-spore forming, rod shaped bacteria which ferment lactose at $35.0 \pm 0.5^{\circ}\text{C}$ in 24-48 hours. Included in this group are the genera *Escherichia*, *Enterobacter*, *Citrobacter* and *Klebsiella*. These organisms are widely distributed in nature and many are native to the gut of warm blooded animals, including man. The term "fecal coliform" refers to the thermotolerant forms of the total coliform group which ferment lactose at $44.5 \pm 0.2^{\circ}$ in 24 hours. Within this group, *Escherichia coli* and *Klebsiella sp.* are of interest since, when present, they indicate that recent fecal contamination has occurred. Enumeration of total coliforms and fecal coliforms using the most probable number method is a means of determining the sanitary quality of sediments.

1.0 Necessary Materials and Equipment

1.1 Glassware, Plasticware, Equipment and Disposable Supplies

- 2 liter Erlenmeyer flasks
- 6 x 50 mm culture tubes
- 16 x 100 mm test tubes
- 16 x 150 mm test tubes
- 16 and test tube caps and racks
- 1.0 and 10.0 ml sterile pipettes
- 20 liter polypropylene carboy
- Heating mantle with magnetic stirrer and stir bars
- Balance and weigh boats
- Autoclave
- Cornwall syringe
- Air incubators $35.0 \pm 0.5^{\circ}\text{C}$
- Waterbaths $44.5 \pm 0.2^{\circ}\text{C}$
- Sterile specimen cups
- Sterile transfer sticks
- Timer

1.0 **Necessary Materials and Equipment (continued)**

1.1 **Glassware, Plasticware, Equipment and Disposable Supplies**

pH meter

1.2 **Media and Diluent**

Lauryl Tryptose Broth

Brilliant Green Bile 2%

EC Medium

Potassium phosphate

Sodium hydroxide

Deionized water

2.0 **Media and Diluent Preparation**

2.1 **Enrichment Media Preparation: Single Strength Lauryl Tryptose Broth**

2.1.1 Weigh 35.6 g of lauryl tryptose broth into a 2 liter Erlenmeyer flask.

2.1.2 Add 1.0 liter of deionized water and bring to a boil with continuous stirring.

2.1.3 Dispense the medium in 10.0 ml aliquots into 16 x 150 mm test tubes containing inverted 6 x 50 mm culture tubes.

2.1.4 Cap the test tubes and autoclave at 121°C for 15 minutes, total time of heat exposure **MUST** not exceed 45 minutes.

2.1.5 Store the media at 4°C in the dark for up to one month.

2.2 **Total Coliform Confirmation Media Preparation: Brilliant Green Bile 2%**

2.2.1 Weigh 40.0 g of Brilliant Green Bile 2% into a 2 liter Erlenmeyer flask.

2.2.2 Add 1.0 liter of deionized water and warm slightly with continuous stirring to completely dissolve the medium.

2.2.3 Dispense the medium in 5.0 ml aliquots into 16 x 100 mm test tubes containing inverted 6 x 50 mm culture tubes.

2.2.4 Cap the test tubes and autoclave at 121°C for 15 minutes, total time of heat exposure **MUST** not exceed 45 minutes.

2.2.5 Store media at 4°C in the dark for up to one month.

2.3 **Fecal Coliform Confirmation Media Preparation: EC Medium**

2.3.1 Weigh 37.0 g of EC medium into a 2 liter Erlenmeyer flask.

2.3.2 Add 1.0 liter of deionized water and bring to a boil with continuous stirring.

2.3.3 Dispense the medium in 5.0 ml aliquots into 16 x 100 mm test tubes containing inverted 6 x 50 mm culture tubes.

2.3.4 Cap the test tubes and autoclave at 121°C for 15 minutes, total time of heat exposure **MUST** not exceed 45 minutes.

2.3.5 Store media at 4°C in the dark for up to one month.

2.0 Media and Diluent Preparation (continued)

2.4 Stock Phosphate Buffer Solution: Buffered Dilution Water

- 2.4.1 In a 1 liter volumetric flask, dissolve 34.0 g of potassium phosphate in 500 ml of deionized water.
- 2.4.2 Adjust to pH 7.2 with 10N sodium hydroxide.
- 2.4.3 Make up to 1 liter with deionized water.
- 2.4.4 Add the buffer solution to a 1 liter polypropylene bottle and autoclave at 121°C for 15 minutes.
- 2.4.5 Store at 4°C in the dark for up to one month.

2.5 Diluent Preparation: Phosphate Buffered Dilution Water

- 2.5.1 Add 20 liters of deionized water to an autoclavable carboy.
- 2.5.2 Add 25 ml of stock phosphate buffer solution and mix thoroughly.
- 2.5.3 Autoclave at 121°C for 45 minutes.
- 2.5.4 Store at room temperature for up to two weeks.

2.6 Diluent Preparation: Phosphate Buffered Dilution Blanks

- 2.6.1 Add 1.0 liter of deionized water to a 2 liter Erlenmeyer flask.
- 2.6.2 Add 1.25 ml of stock phosphate buffer solution and mix thoroughly.
- 2.6.3 Dispense the medium in 9.2 ml aliquots into 16 x 150 mm test tubes, 81.0 ml aliquots into 100 ml graduated dilution bottles and 100.0 ml aliquots into 100 ml graduated dilution bottles.
- 2.6.4 Cap the test tubes and/or dilution bottles and autoclave at 121°C for 15 minutes.
- 2.6.5 Refrigerate at 4°C in the dark for up to one month. **NOTE: Always check the volume of dilution water present in dilution blanks with each use. Discard any batch of buffered dilution water where evaporation has resulted in an inappropriate volume of buffered dilution water.**

3.0 Sample Storage

3.1 Sediment Storage Containers

- 3.1.1 Use containers for sediment which are waterproof and durable enough to withstand the cutting action of sharp sediment particles and abrasion during transport. Waterproof paper bags and plastic bags are suitable containers.

3.2 Sediment Storage Conditions and Temperature

- 3.2.1 Keep sediment samples in dry storage at a temperature above freezing but lower than 10°C until examination. Do not allow samples to come in contact with ice.

3.0 Sample Storage (continued)

3.3 Sediment Identification Records

- 3.3.1 Maintain records of identification for each sediment sample to enter the laboratory. Include the date, time, and location of collection, and the physical and chemical measurements of the environment (if applicable).
- 3.3.2 Mark individual containers of sediment samples for identification. Also, include identification information on a descriptive form to accompany the sample.
- 3.3.3 Record the time elapsed between collection and examination for each sediment sample examined in the laboratory. Examine samples within 6 hours after collection and in no case examine sediment if they have been held more than 24 hours after collection.

4.0 Sample Preparation

- 4.1 Use a sterile tongue depressor to homogenize sediment samples in their respective collection containers.
- 4.2 Note the consistency of the sediment (mud, sand, rocks) on the data sheet.
- 4.3 Weigh 20 g of the homogenized sediment sample into a sterile specimen cup.
- 4.4 Add 180 ml of sterile phosphate buffered dilution water to the cup.
- 4.5 Alcohol and flame a magnetic stir bar before adding it to the sediment and buffer mixture.
- 4.6 Mix the sediment and buffer for 2 minutes prior to transfer to media or dilution blanks.

5.0 Sample Analyses

5.1 Presumptive Test Using Lauryl Tryptose Broth

- 5.1.1 Inoculate each of 5 single strength lauryl tryptose broth tubes with 10.0 ml of the prepared sample (equal to 1.0 g of sediment), 5 single strength lauryl tryptose broth tubes with 1.0 ml of a 10 fold dilution of the prepared sample (equal to 0.1 g of sediment) and 5 single strength lauryl tryptose broth tubes with 1.0 ml of a 100 fold dilution of the prepared sample (equal to 0.01 g of sediment).
- 5.1.2 In order to avoid indeterminate results, perform extensions of the dilutions mentioned.
- 5.1.3 Incubate tubes at $35.0 \pm 0.5^\circ\text{C}$ for 24 ± 2 hours.
- 5.1.4 Examine tubes for the presence/absence of gas production.
- 5.1.5 Score tubes with gas production and perform confirmation tests at this time.
- 5.1.6 Return tubes to $35.0 \pm 0.5^\circ\text{C}$.

5.0 Sample Analyses (continued)

- 5.1.7 Re-examine the tubes at 48 ± 3 hours for the presence/absence of gas production.
- 5.1.8 Score tubes with gas production and perform confirmation tests at this time.
- 5.1.9 Absence of gas production at the end of 48 ± 3 hours constitutes a negative test result for the total and fecal coliform groups.

5.2 Confirmed Tests Using Brilliant Green Bile 2% and EC Medium

- 5.2.1 General Rules for Performing the Confirmed Test (when 3 or more replicate portions of a series of 3 or more decimal dilutions of sample are planted): Select the tubes of the highest dilution (smallest volume) in which all tubes show gas production in 24 hours. Submit all of these tubes as well as every one of the gas-positive tubes in all higher dilutions to the confirmed tests. If all tubes show gas production, submit all tubes of the highest dilution and of the next-to-the-highest dilution to the confirmed tests. Submit all tubes of all dilutions in which gas is produced only at the end of 48 hours incubation to the confirmed test. All tubes producing gas that have not been submitted to the confirmed test shall be recorded as containing organisms of the coliform group, even though all the confirmed tests may yield negative results.
- 5.2.2 Mix the presumptive lauryl tryptose broth tubes by gentle shaking or rotating.
- 5.2.3 Transfer sample using sterile transfer sticks from lauryl tryptose broth tubes to Brilliant Green Bile 2% and EC Medium tubes.
- 5.2.4 Incubate Brilliant Green Bile tubes at $35.0 \pm 0.5^\circ\text{C}$ for 48 ± 3 hours and EC Medium tubes at $44.5 \pm 0.2^\circ\text{C}$ for 48 ± 3 hours.
- 5.2.5 Examine tubes for the presence/absence of gas production.
- 5.2.6 Score Brilliant Green Bile 2% tubes with gas production positive for the total coliform group and EC Medium tubes with gas production positive for the fecal coliform group.
- 5.2.7 Score Brilliant Green Bile 2% tubes and EC Medium tubes with no gas production negative for the total and fecal coliform groups.

6.0 Using the Most Probable Number Geometric Series

- 6.1 Count the number of positive tubes from each of the three dilutions performed.
- 6.2 Use the number of positive tubes from each dilution to form a 3 digit code.
- 6.3 Locate your three digit code on the most probable number geometric series chart.
- 6.4 Read the MPN number which corresponds to your three digit code. This is your density per 100 g of sample.

6.0 Using the Most Probable Number Geometric Series (continued)

6.5 If, instead of 10, 1.0, and 0.1 ml a combination of portions of 100, 10, and 1 ml is used, record the MPN as 0.1 times the figure in the tabulation. On the other hand, should a combination of corresponding portions of 1.0, 0.1 and 0.01 ml be planted, record 10 times the figure in the tabulation; should a combination of portions of 0.1, 0.01, and 0.001 ml be planted, record 100 times the figure in the tabulation.

6.6 When more than 3 dilutions are employed in a decimal series of dilutions, the results from only 3 of them are significant. To select the 3 dilutions to be used in determining the MPN index, using the system of 5 tubes of each dilutions as an example, choose the highest dilution which gives positive results in all of the 5 portions tested (no lower dilution giving any negative results) and the 2 next succeeding high dilutions. The results of these 3 volumes should then be used in the computations of the MPN index.

In examples given below, significant dilutions results are shown in boldface. The number in the numerator represents positive tubes; that in the denominator, the total tubes planted.

	1 ml or g	0.1 ml or g	0.01 ml or g	0.001 ml or g
(a)	5/5	5/5	2/5	0/5
(b)	5/5	4/5	2/5	0/5
(c)	0/5	1/5	0/5	0/5
(d)	5/5	3/5	1/5	1/5
(e)	5/5	3/5	2/5	0/5

In example (c) above, the first 3 dilutions are taken in order to throw the positive result into the middle dilution. When a case arises such as is shown in (d), where a positive occurs in a dilution higher than the 3 chosen according to the rule, it is included in the result of the highest chosen dilution (e).

7.0 Trouble Shooting

7.1 Thoroughly homogenize sediments before preparing dilutions.

7.2 Adequately mix samples and dilutions of samples prior to addition to enrichment tubes.

7.3 Adequately mix presumptive enrichment tubes prior to addition of sample to confirmation enrichment tubes.

8.0 Statistical Analysis and Data Usage

8.1 Tabulate and summarize the data.

8.2 Use data in conjunction with other indicator tests to determine sanitary quality.

Environmental Testing Center
Standard Operating Procedure
MPN Sediment
Total and Fecal Coliforms
October 1994

9.0 **References**

- 9.1 American Public Health Association, Inc. 1970. Recommended Procedures for the Examination of Sea Water and Shellfish. Washington, DC. p. 1-105.
- 9.2 American Public Health Association, American Water Works Association, Water Pollution Control Federation. 1989. Standard Methods for the Examination of Water and Wastewater, Seventeenth Edition. Washington, DC. Chapter 9.

MOST PROBABLE NUMBER METHOD FOR THE ENUMERATION OF *CLOSTRIDIUM PERFRINGENS* IN SHELLFISH

Point of Contact

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Clostridium perfringens is a plump, nonmotile, gram-positive, anaerobic rod. This microorganism is often found in the intestines of humans and animals as part of the normal microbiota. This spore-forming microorganism is capable of surviving for long periods of time in the environment, and thus is often used in the detection of fecal contamination that is several years old. Enumeration of *Clostridium perfringens* using the most probable number method is a means of determining the sanitary quality of shellfish meats.

1.0 Necessary Materials and Equipment

1.1 Glassware, Plasticware, Equipment and Disposable Supplies

- 2 liter Erlenmeyer flasks
- 9 x 50 mm sterile petri dishes
- 16 x 150 mm test tubes
- 20 x 150 mm test tubes
- 16 and 20 mm test tube caps
- 16 and 20 mm test tube racks
- 1.0 and 10.0 ml sterile pipettes
- Sterile transfer sticks
- Heating mantle with magnetic stirrer and stir bars
- Balance and weigh boats
- Autoclave
- pH meter
- Cornwall syringe
- Air incubators $45.0 \pm 0.5^{\circ}\text{C}$
- Waterbath $44-46^{\circ}\text{C}$
- Alcohol burner
- Membranes, 0.45 micron
- Membrane forceps
- Anaerobic gaspack with gaspacks, indicator strips and catalyst
- Sterile shucking knives
- Sterile scrub brushes

1.0 Necessary Materials and Equipment (continued)

Waring blenders

Timer

1.2 Media and Diluent

Homogenized milk

Iron filings (fine grain)

Bacteriological grade agar

Tryptose

Yeast extract

Sucrose

L-cysteine hydrochloride

Bromocresol purple

Magnesium sulfate

Indoxyl-B-D-glucoside (IBDG)

D-Cycloserine

Polymyxin B sulfate

Ferric chloride

Phenolphthalein diphosphate

Ammonium hydroxide

Potassium phosphate

Sodium hydroxide

Deionized water

2.0 Media and Diluent Preparation

2.1 Enrichment Media Preparation: Single Strength Iron Milk Media

2.1.1 Iron milk media is prepared fresh, the day of the sample analysis.

2.1.2 Determine the number of test tubes of media to prepare by multiplying the number of samples to be analyzed by fifteen.

2.1.3 Add 0.2 g of iron filings to each 16 x 150 mm test tube.

2.1.4 Add 10.0 ml of homogenized milk to each test tube.

2.1.5 Cover each of the test tube racks with a sheet of aluminum foil and autoclave at 116°C for 10 minutes.

2.1.6 Temper the media to 44-46°C approximately 1 hour prior to assay.

2.2 Enrichment Media Preparation: Double Strength Iron Milk Media

2.2.1 Iron milk media is prepared fresh, the day of the sample analysis.

2.2.2 Determine the number of test tubes of media to prepare by multiplying the number of samples to be analyzed by five.

2.2.3 Add 0.4 g of iron filings to each 20 x 150 mm test tube.

2.2.4 Add 20.0 ml of homogenized milk to each test tube.

2.2.5 Cover each of the test tube racks with a sheet of aluminum foil and autoclave at 116°C for 10 minutes.

2.0 Media and Diluent Preparation (continued)

2.2.6 Temper the media to 44-46°C approximately 1 hour prior to assay.

2.3 Confirmation Media Preparation: Membrane Filtration Media (mCP)

2.3.1 Weigh 30.0 g of tryptose, 20.0 g of yeast extract, 5.0 g of sucrose, 1.0 g of L-cysteine hydrochloride, 0.1 g of magnesium sulfate, 0.04 g of bromocresol purple and 15.0 g of agar into a 2 liter Erlenmeyer flask. Add 900.0 ml of deionized water and mix well.

2.3.2 Heat with continuous mixing. Boil for approximately 1 minute to completely dissolve the powder.

2.3.3 Adjust the pH to 7.6 using 10N NaOH.

2.3.4 Autoclave at 121°C for 15 minutes.

2.3.5 Temper the media to 44-46°C for approximately 30 minutes.

2.3.6 Aseptically add:

0.4 g of D-cycloserine

0.025 g of polymyxin B sulfate

0.06 g of IBDG

2.0 ml of a 4.5% ferric chloride solution

20.0 ml of a 0.5% phenolphthalein solution

2.3.7 Dispense the medium into 9 x 50 mm petri dishes in 4.5 ml aliquots and allow to solidify.

2.3.8 Store mCP agar plates inverted at 4°C in the dark for up to one month.

2.4 Stock Phosphate Buffer Solution: Buffered Dilution Water

2.4.1 In a 1 liter polypropylene bottle, dissolve 34.0 g of potassium phosphate in 500 ml of deionized water.

2.4.2 Adjust to pH 7.2 with 10N sodium hydroxide.

2.4.3 Make up to 1 liter with deionized water.

2.4.4 Autoclave at 121°C for 15 minutes.

2.4.5 Store at 4°C in the dark for up to one month.

2.5 Diluent Preparation: Phosphate Buffered Dilution Water

2.5.1 Add 20 liters of deionized water to a 20 liter autoclavable carboy.

2.5.2 Add 25 ml of stock phosphate buffer solution.

2.5.3 Autoclave at 121°C for 45 minutes.

2.5.4 Store at room temperature for up to one month.

2.6 Diluent Preparation: Phosphate Buffered Dilution Blanks

2.6.1 Add 1.0 liter of deionized water to a 2 liter Erlenmeyer flask.

2.6.2 Add 1.25 ml of stock phosphate buffer solution.

2.6.3 Dispense the medium in 9.2 ml aliquots into 16 x 150 mm test tubes, 81.0 ml aliquots into 100 ml graduated dilution bottles and 100.0 ml aliquots into 100 ml graduated dilution bottles.

2.6.4 Cap the test tubes and/or dilution bottles and autoclave at 121°C for 15 minutes.

2.0 Media and Diluent Preparation (continued)

- 2.6.5 Refrigerate at 4°C in the dark for up to one month. **NOTE: Always check the volume of dilution water present in dilution blanks with each use. Discard any batch of buffered dilution water where evaporation has resulted in an inappropriate volume of buffered dilution water.**

3.0 Sample Storage

3.1 Shellfish Storage Containers

- 3.1.1 Use containers for shell-stock which are waterproof and durable enough to withstand the cutting action of the shellfish and abrasion during transport. Waterproof paper bags, paraffined cardboard cups or plastic bags are suitable containers.
- 3.1.2 Use sterile wide-mouth jars with watertight closure for freshly shucked shellfish samples. Transfer samples of the final product to the sample jar with sterile forceps or spoon.
- 3.1.3 Use one or two packages of frozen shucked shellfish, containing 10 to 12 animals each, as one sample. Transfer core samples taken from larger blocks to sterile wide-mouth jars for transportation to the laboratory.

3.2 Shellfish Storage Conditions and Temperature

- 3.2.1 Keep shell-stock samples in dry storage at a temperature above freezing but lower than 10°C until examination. Do not allow samples to come in contact with ice.
- 3.2.2 Refrigerate samples of shucked shellfish immediately after collection by packing in crushed ice until examination.
- 3.2.3 Keep samples of frozen shucked shellfish in the frozen state or at temperatures close to those at which the commercial stock was maintained. When this is not possible, pack samples of frozen shucked shellfish in crushed ice until examination.

3.3 Shellfish Identification Records

- 3.3.1 Maintain records of identification for each shellfish sample to enter the laboratory. Include the date, time, and place of collection, area the shellfish were harvested, the date and time of harvesting and the physical and chemical measurements of the harvesting water (if applicable). Also include the storage conditions between harvesting and collection.
- 3.3.2 Mark individual containers of shellfish samples for identification. Also, include identification information on a descriptive form to accompany the sample.

3.0 Sample Storage (continued)

- 3.3.3 Record the time elapsed between collection and examination for each shellfish sample examined in the laboratory. Examine samples of shell stock and shucked, unfrozen shellfish within 6 hours after collection and in no case examine shellfish if they have been held more than 24 hours after collection.
- 3.3.4 Record the identification of the shipper, the date of shipment, and the harvesting area as well as the date, time and place of collection for shellfish samples collected in market areas and when other specified information is not available.

4.0 Sample Preparation

4.1 Shellfish in the Shell - Cleaning the Shells

- 4.1.1 Use a minimum of 12 shellfish in order to obtain a representative sample (approximately 200 g of shellfish liquor and meats) and to allow for the selection of sound animals suitable for shucking. Collect at least 200 g of sample when examining shucked or frozen shellfish.
- 4.1.2 Scrub hands thoroughly with soap and water.
- 4.1.3 Scrape off all growth and loose material from the shell. Scrub the shell stock with a sterile stiff brush under running water of drinking-water quality, paying particular attention to the crevices at the junctions of the shells.
- 4.1.4 Place the cleaned shell stock in clean containers or on clean towels to drain in the air.

4.2 Removal of Shell Contents

- 4.2.1 Before starting the removal of shell contents, scrub hands thoroughly with soap and water and rinse with 70% alcohol.
- 4.2.2 **OYSTERS:** Hold the oyster in the hand or on a fresh clean paper towel on the bench with the deep shell on the bottom. Using a sterile oyster knife, insert the point between the shells on the ventral side (at the right when the hinge is pointed away from the examiner), about 1/4 the distance from the hinge to the bill. Entry may also be made at the bill. Cut the adductor muscle from the upper flat shell and pry the shell wide enough to drain the shell liquor into a sterile tared blender. The upper shell may then be pried loose at the hinge, discarded, and the meats transferred to a sterile tared blender after severing the muscle attachment to the lower shell.

4.0 Sample Preparation (continued)

- 4.2.3 **HARD CLAMS:** Enter the hard clam with a sterile, thin-bladed knife similar to a paring knife. To open the clam, hold it in the hand, place the edge of the knife at the junction of the bills, and force it between the shells with a squeezing motion. An alternative method is to nibble a small hole in the bill, and with the knife, sever the two adductor muscles. Drain the shell liquor into a sterile tared blender. Cut the adductor muscles from the shells and transfer the body of the animal to the blender.
- 4.2.4 **OTHER CLAMS:** Shuck soft clams, butter clams, surf clams and similar species with a sterile paring knife, entering at the siphon end and cutting the adductor muscles first from the top valve and then from the bottom valve. Drain the shell liquor into a sterile tared blender. Transfer the body of the animal to the blender.
- 4.2.5 **MUSSELS:** Remove the byssal threads during the cleansing of the shell. Enter the mussel at the byssal opening. Insert the knife and spread the shells apart with a twisting motion, draining the liquor of the shellfish into a sterile tared blender. Transfer the body of the animal to the blender.

4.3 Dilution and Grinding

- 4.3.1 Weigh the shellfish meats and liquors.
- 4.3.2 Add an equal amount, by weight, of phosphate buffered dilution water to the blender.
- 4.3.3 Grind for 60-120 sec in a sterile waring blender operating at approximately 14,000 RPM. Avoid excessive grinding to prevent overheating.

5.0 Sample Analyses

5.1 Presumptive Test Using Iron Milk Media

- 5.1.1 Inoculate each of 5 double strength iron milk media tubes with 2.0 ml of the prepared sample (equal to 1.0 g of shellfish), 5 single strength iron milk media tubes with 1.0 ml of a 10 fold dilution of the prepared sample (equal to 0.1 g of shellfish) and 5 single strength iron milk media tubes with 1.0 ml of a 100 fold dilution of the prepared sample (equal to 0.01 g of shellfish).
- 5.1.2 In order to avoid indeterminate results, perform extensions of the dilutions mentioned.
- 5.1.3 Incubate tubes at $45.0 \pm 0.5^\circ\text{C}$ for 18 hours.
- 5.1.4 Examine tubes for the presence/absence of stormy fermentation/gas production.

5.0 Sample Analyses (continued)

5.1.5 Score tubes with stormy fermentation/gas production positive for presence of *Clostridium perfringens*.

5.2 *Clostridium perfringens* Confirmation Test Using mCP Agar

5.2.1 Mix the presumptive iron milk media tubes by gentle shaking

5.2.2 Place a sterile 0.45 micron membrane on a mCP agar plate.

5.2.3 Transfer sample using sterile transfer sticks from iron milk media tubes with stormy fermentation/gas production to the membrane of a mCP agar plate.

5.2.4 Repeat this process for each tube with stormy fermentation/gas production. Use one plate for each dilution (maximum 5 transfers/plate).

5.2.5 Incubate the inverted plates anaerobically at $45.0 \pm 0.5^\circ\text{C}$ for 18-24 hours.

5.2.6 After the incubation period, score all presumptive *Clostridium perfringens* transfers (yellow to greenish in color).

5.2.7 Hold plates over fresh ammonium hydroxide for approximately 20-30 seconds.

5.2.8 Score *Clostridium perfringens* transfers, which turn magenta.

6.0 Using the Most Probable Number Geometric Series

6.1 Count the number of positive tubes from each of the three dilutions performed.

6.2 Use the number of positive tubes from each dilution to form a 3 digit code.

6.3 Locate your three digit code on the most probable number geometric series chart.

6.4 Read the MPN number which corresponds to your three digit code. This is your density per 100 g of sample.

6.5 If, instead of 10, 1.0, and 0.1 ml a combination of portions of 100, 10, and 1 ml is used, record the MPN as 0.1 times the figure in the tabulation. On the other hand, should a combination of corresponding portions of 1.0, 0.1 and 0.01 ml be planted, record 10 times the figure in the tabulation; should a combination of portions of 0.1, 0.01, and 0.001 ml be planted, record 100 times the figure in the tabulation.

6.6 When more than 3 dilutions are employed in a decimal series of dilutions, the results from only 3 of them are significant. To select the 3 dilutions to be used in determining the MPN index, using the system of 5 tubes of each dilutions as an example, choose the highest dilution which gives positive results in all of the 5 portions tested (no lower dilution giving any negative results) and the 2 next succeeding high dilutions. The results of these 3 volumes should then be used in the computations of the MPN index. In examples given below, significant dilutions results are shown in boldface.

6.0 Using the Most Probable Number Geometric Series (continued)

The number in the numerator represents positive tubes; that in the denominator, the total tubes planted.

	1 ml or g	0.1 ml or g	0.01 ml or g	0.001 ml or g
(a)	5/5	5/5	2/5	0/5
(b)	5/5	4/5	2/5	0/5
(c)	0/5	1/5	0/5	0/5
(d)	5/5	3/5	1/5	1/5
(e)	5/5	3/5	2/5	0/5

In example (c) above, the first 3 dilutions are taken in order to throw the positive result into the middle dilution. When a case arises such as is shown in (d), where a positive occurs in a dilution higher than the 3 chosen according to the rule, it is included in the result of the highest chosen dilution (e).

7.0 Trouble Shooting

- 7.1 Thoroughly clean the shells of each shellfish, especially the hinge regions.
- 7.2 Adequately mix samples and dilutions of samples prior to addition to enrichment tubes.
- 7.3 Adequately mix presumptive enrichment tubes prior to transfer of sample to confirmation media.

8.0 Statistical Analysis and Data Usage

- 8.1 Tabulate and summarize the data.
- 8.2 Use data in conjunction with other indicator tests to determine sanitary quality.

9.0 References

- 9.1 American Public Health Association, Inc. 1970. Recommended Procedures for the Examination of Sea Water and Shellfish. Washington, DC. p. 1-105.
- 9.2 American Public Health Association, American Water Works Association, Water Pollution Control Federation. 1989. Standard Methods for the Examination of Water and Wastewater, Seventeenth Edition. Washington, DC. Chapter 9.
- 9.3 St. John, W.D., J.R. Matches and M.M. Wekell. 1982. Use of Iron Milk Medium for Enumeration of *Clostridium perfringens*. J. Assoc. Off. Anal. Chem. 65:1129-1133.
- 9.4 Bisson, J.W., V.J. Cabelli. 1978. Membrane filter enumeration method for *Clostridium perfringens*. J. Appl. Environ. Microbiol. 37:55-66.
- 9.5 Millipore Corporation. 1992. Water Microbiology/Laboratory and Field Procedures. Bedford, MA. p. 1-56.

MOST PROBABLE NUMBER METHOD FOR THE ENUMERATION OF *CLOSTRIDIUM PERFRINGENS* IN SEDIMENT

Point of Contact

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Clostridium perfringens is a plump, nonmotile, gram-positive, anaerobic rod. This microorganism is often found in the intestines of humans and animals as part of the normal microbiota. This spore-forming microorganism is capable of surviving for long periods of time in the environment, and thus is often used in the detection of fecal contamination that is several years old. Enumeration of *Clostridium perfringens* using the most probable number method is a means of determining the sanitary quality of shellfish meats.

1.0 Necessary Materials and Equipment

1.1 Glassware, Plasticware, Equipment and Disposable Supplies

- 2 liter Erlenmeyer flasks
- 9 x 50 mm sterile petri dishes
- 16 x 150 mm test tubes
- 20 x 150 mm test tubes
- 16 and 20 mm test tube caps
- 16 and 20 mm test tube racks
- 1.0 and 10.0 ml sterile pipettes
- Sterile transfer sticks
- Heating mantle with magnetic stirrer and stir bars
- Balance and weigh boats
- Autoclave
- pH meter
- Cornwall syringe
- Air incubators $45.0 \pm 0.5^{\circ}\text{C}$
- Waterbath $44-46^{\circ}\text{C}$
- Alcohol burner
- Membranes, 0.45 micron
- Membrane forceps
- Anaerobic gaspack with gaspacks, indicator strips and catalyst
- Sterile specimen cups
- Timer

1.0 Necessary Materials and Equipment (continued)

1.2 Media and Diluent

Homogenized milk
Iron filings (fine grain)
Bacteriological grade agar
Tryptose
Yeast extract
Sucrose
L-cysteine hydrochloride
Bromocresol purple
Magnesium sulfate
Indoxyl-B-D-glucoside (IBDG)
D-Cycloserine
Polymyxin B sulfate
Ferric chloride
Phenolphthalein diphosphate
Ammonium hydroxide
Potassium phosphate
Sodium hydroxide
Deionized water

2.0 Media and Diluent Preparation

2.1 Enrichment Media Preparation: Single Strength Iron Milk Media

- 2.1.1 Iron milk media is prepared fresh, the day of the sample analysis.
2.1.2 Determine the number of test tubes of media to prepare by multiplying the number of samples to be analyzed by fifteen.
2.1.3 Add 0.2 g of iron filings to each 16 x 150 mm test tube.
2.1.4 Add 10.0 ml of homogenized milk to each test tube.
2.1.5 Cover each of the test tube racks with a sheet of aluminum foil and autoclave at 116°C for 10 minutes.
2.1.6 Temper the media to 44-46°C approximately 1 hour prior to assay.

2.2 Enrichment Media Preparation: Double Strength Iron Milk Media

- 2.2.1 Iron milk media is prepared fresh, the day of the sample analysis.
2.2.2 Determine the number of test tubes of media to prepare by multiplying the number of samples to be analyzed by five.
2.2.3 Add 0.4 g of iron filings to each 20 x 150 mm test tube.
2.2.4 Add 20.0 ml of homogenized milk to each test tube.
2.2.5 Cover each of the test tube racks with a sheet of aluminum foil and autoclave at 116°C for 10 minutes.
2.2.6 Temper the media to 44-46°C approximately 1 hour prior to assay.

2.0 Media and Diluent Preparation (continued)

2.3 Confirmation Media Preparation: Membrane Filtration Media (mCP)

- 2.3.1 Weigh 30.0 g of tryptose, 20.0 g of yeast extract, 5.0 g of sucrose, 1.0 g of L-cysteine hydrochloride, 0.1 g of magnesium sulfate, 0.04 g of bromocresol purple and 15.0 g of agar into a 2 liter Erlenmeyer flask. Add 900.0 ml of deionized water and mix well.
- 2.3.2 Heat with continuous mixing. Boil for approximately 1 minute to completely dissolve the powder.
- 2.3.3 Adjust the pH to 7.6 using 10N NaOH.
- 2.3.4 Autoclave at 121°C for 15 minutes.
- 2.3.5 Temper the media to 44-46°C for approximately 30 minutes.
- 2.3.6 Aseptically add:
 - 0.4 g of D-cycloserine
 - 0.025 g of polymyxin B sulfate
 - 0.06 g of IBDG
 - 2.0 ml of a 4.5% ferric chloride solution
 - 20.0 ml of a 0.5% phenolphthalein solution
- 2.3.7 Dispense the medium into 9 x 50 mm petri dishes in 4.5 ml aliquots and allow to solidify.
- 2.3.8 Store mCP agar plates inverted at 4°C in the dark for up to one month.

2.4 Stock Phosphate Buffer Solution: Buffered Dilution Water

- 2.4.1 In a 1 liter polypropylene bottle, dissolve 34.0 g of potassium phosphate in 500 ml of deionized water.
- 2.4.2 Adjust to pH 7.2 with 10N sodium hydroxide.
- 2.4.3 Make up to 1 liter with deionized water.
- 2.4.4 Autoclave at 121°C for 15 minutes.
- 2.4.5 Store at 4°C in the dark for up to one month.

2.5 Diluent Preparation: Phosphate Buffered Dilution Water

- 2.5.1 Add 20 liters of deionized water to a 20 liter autoclavable carboy.
- 2.5.2 Add 25 ml of stock phosphate buffer solution.
- 2.5.3 Autoclave at 121°C for 45 minutes.
- 2.5.4 Store at room temperature for up to one month.

2.6 Diluent Preparation: Phosphate Buffered Dilution Blanks

- 2.6.1 Add 1.0 liter of deionized water to a 2 liter Erlenmeyer flask.
- 2.6.2 Add 1.25 ml of stock phosphate buffer solution.
- 2.6.3 Dispense the medium in 9.2 ml aliquots into 16 x 150 mm test tubes and 91.0 ml aliquots into 100 ml graduated dilution bottles.
- 2.6.4 Cap the test tubes and/or dilution bottles and autoclave at 121°C for 15 minutes.

2.0 Media and Diluent Preparation (continued)

- 2.6.5 Refrigerate at 4°C in the dark for up to one month. **NOTE:** Always check the volume of dilution water present in dilution blanks with each use. Discard any batch of buffered dilution water where evaporation has resulted in an inappropriate volume of buffered dilution water.

3.0 Sample Storage

3.1 Sediment Storage Containers

- 3.1.1 Use containers for sediment which are waterproof and durable enough to withstand the cutting action of sharp sediment particles and abrasion during transport. Waterproof paper bags, paraffined cardboard cups or plastic bags are suitable containers.

3.2 Sediment Storage Conditions and Temperature

- 3.2.1 Keep sediment samples in dry storage at a temperature above freezing but lower than 10°C until examination. Do not allow samples to come in contact with ice.

3.3 Sediment Identification Records

- 3.3.1 Maintain records of identification for each sediment sample to enter the laboratory. Include the date, time, and location of collection, and the physical and chemical measurements of the environment (if applicable).
- 3.3.2 Mark individual containers of sediment samples for identification. Also, include identification information on a descriptive form to accompany the sample.
- 3.3.3 Record the time elapsed between collection and examination for each sediment sample examined in the laboratory. Examine samples within 6 hours after collection and in no case examine sediment if they have been held more than 24 hours after collection.

4.0 Sample Preparation

- 4.1 Use a sterile tongue depressor to homogenize sediment samples in their respective collection containers.
- 4.2 Note the consistency of the sediment (mud, sand, rocks) on the data sheet.
- 4.3 Weigh 20 g of the homogenized sediment sample into a sterile specimen cup.
- 4.4 Add 180 ml of sterile phosphate buffered dilution water to the cup.
- 4.5 Alcohol and flame a magnetic stir bar before adding it to the sediment and buffer mixture.
- 4.6 Mix the sediment and buffer for 2 minutes prior to transfer to media or dilution blanks.

5.0 Sample Analyses

5.1 Presumptive Test Using Iron Milk Media

- 5.1.1 Inoculate each of 5 double strength iron milk media tubes with 10.0 ml of the prepared sample (equal to 1.0 g of sediment), 5 single strength iron milk media tubes with 1.0 ml of the prepared sample (equal to 0.1 g of sediment) and 5 single strength iron milk media tubes with 1.0 ml of a 10 fold dilution of the prepared sample (equal to 0.01 g of sediment).
- 5.1.2 In order to avoid indeterminate results, perform extensions of the dilutions mentioned.
- 5.1.3 Incubate tubes at $45.0 \pm 0.5^\circ\text{C}$ for 18 hours.
- 5.1.4 Examine tubes for the presence/absence of stormy fermentation/gas production.
- 5.1.5 Score tubes with stormy fermentation/gas production positive for presence of *Clostridium perfringens*.

5.2 *Clostridium perfringens* Confirmation Test Using mCP Agar

- 5.2.1 Mix the presumptive iron milk media tubes by gentle shaking
- 5.2.2 Place a sterile 0.45 micron membrane on a mCP agar plate.
- 5.2.3 Transfer sample using sterile transfer sticks from iron milk media tubes with stormy fermentation/gas production to the membrane of a mCP agar plate.
- 5.2.4 Repeat this process for each tube with stormy fermentation/gas production. Use one plate for each dilution (maximum 5 transfers/plate).
- 5.2.5 Incubate the inverted plates anaerobically at $45.0 \pm 0.5^\circ\text{C}$ for 18-24 hours.
- 5.2.6 After the incubation period, score all presumptive *Clostridium perfringens* transfers (yellow to greenish in color).
- 5.2.7 Hold plates over fresh ammonium hydroxide for approximately 20-30 seconds.
- 5.2.8 Score *Clostridium perfringens* transfers, which turn magenta.

6.0 Using the Most Probable Number Geometric Series

- 6.1 Count the number of positive tubes from each of the three dilutions performed.
- 6.2 Use the number of positive tubes from each dilution to form a 3 digit code.
- 6.3 Locate your three digit code on the most probable number geometric series chart.
- 6.4 Read the MPN number which corresponds to your three digit code. This is your density per 100 g of sample.

6.0 Using the Most Probable Number Geometric Series (continued)

6.5 If, instead of 10, 1.0, and 0.1 ml a combination of portions of 100, 10, and 1 ml is used, record the MPN as 0.1 times the figure in the tabulation. On the other hand, should a combination of corresponding portions of 1.0, 0.1 and 0.01 ml be planted, record 10 times the figure in the tabulation; should a combination of portions of 0.1, 0.01, and 0.001 ml be planted, record 100 times the figure in the tabulation.

6.6 When more than 3 dilutions are employed in a decimal series of dilutions, the results from only 3 of them are significant. To select the 3 dilutions to be used in determining the MPN index, using the system of 5 tubes of each dilutions as an example, choose the highest dilution which gives positive results in all of the 5 portions tested (no lower dilution giving any negative results) and the 2 next succeeding high dilutions. The results of these 3 volumes should then be used in the computations of the MPN index. In examples given below, significant dilutions results are shown in boldface.

The number in the numerator represents positive tubes; that in the denominator, the total tubes planted.

	1 ml or g	0.1 ml or g	0.01 ml or g	0.001 ml or g
(a)	5/5	5/5	2/5	0/5
(b)	5/5	4/5	2/5	0/5
(c)	0/5	1/5	0/5	0/5
(d)	5/5	3/5	1/5	1/5
(e)	5/5	3/5	2/5	0/5

In example (c) above, the first 3 dilutions are taken in order to throw the positive result into the middle dilution. When a case arises such as is shown in (d), where a positive occurs in a dilution higher than the 3 chosen according to the rule, it is included in the result of the highest chosen dilution (e).

7.0 Trouble Shooting

7.1 Thoroughly homogenize sediments before preparing dilutions.

7.2 Adequately mix samples and dilutions of samples prior to addition to enrichment tubes.

7.3 Adequately mix presumptive enrichment tubes prior to transfer of sample to confirmation media.

8.0 Statistical Analysis and Data Usage

8.1 Tabulate and summarize the data.

8.2 Use data in conjunction with other indicator tests to determine sanitary quality.

9.0 References

- 9.1 American Public Health Association, Inc. 1970. Recommended Procedures for the Examination of Sea Water and Shellfish. Washington, DC. p. 1-105.
- 9.2 American Public Health Association, American Water Works Association, Water Pollution Control Federation. 1989. Standard Methods for the Examination of Water and Wastewater, Seventeenth Edition. Washington, DC. Chapter 9.
- 9.3 St. John, W.D., J.R. Matches and M.M. Wekell. 1982. Use of Iron Milk Medium for Enumeration of *Clostridium perfringens*. J. Assoc. Off. Anal. Chem. 65:1129-1133.
- 9.4 Bisson, J.W., V.J. Cabelli. 1978. Membrane filter enumeration method for *Clostridium perfringens*. J. Appl. Environ. Microbiol. 37:55-66.
- 9.5 Millipore Corporation. 1992. Water Microbiology/Laboratory and Field Procedures. Bedford, MA. p. 1-56.

MOST PROBABLE NUMBER METHOD FOR THE ENUMERATION OF FECAL STREPTOCOCCI AND ENTEROCOCCI IN SHELLFISH

Point of Contact

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The term "fecal streptococci" refers to the Lancefield Group D bacteria. The term "enterococci" refers to a specific subgroup of fecal streptococci including *Streptococcus faecalis*, *Streptococcus faecium*, *Streptococcus avium* and *Streptococcus gallinarum*. Fecal streptococci and enterococci are found in the intestines of humans and other animals, where they behave as commensals of limited pathogenic potential. Because these organisms are associated with fecal wastes of man and animals, they can be isolated from contaminated waters containing such waste. Enumeration of fecal streptococci and enterococci *sp.* using the most probable number method is a means of determining the sanitary quality of shellfish meats.

1.0 Necessary Materials and Equipment

1.1 Glassware, Plasticware, Equipment and Disposable Supplies

- 2 liter Erlenmeyer flasks
- 16 x 150 mm test tubes
- 20 x 150 mm test tubes
- 16 and 20 mm test tube caps
- 16 and 20 mm test tube racks
- 1.0 and 10.0 ml sterile pipettes
- 20 liter polypropylene carboy
- Heating mantle with magnetic stirrer and stir bars
- Balance and weigh boats
- Autoclave
- Cornwall syringe
- Air incubators $35.0 \pm 0.5^{\circ}\text{C}$
- Air incubator $41.0 \pm 0.5^{\circ}\text{C}$
- Waterbath $44-46^{\circ}\text{C}$
- Sterile shucking knives
- Sterile scrub brushes
- Waring blenders

1.0 Necessary Materials and Equipment (continued)

1.1 Glassware, Plasticware, Equipment and Disposable Supplies

Timer
Sterile transfer sticks
pH meter
Alcohol burner
Membranes, 0.45 micron
Membrane forceps

1.2 Media and Diluent

Azide dextrose broth
Ethyl Violet Azide (EVA) Broth
mE agar
Esculin iron agar
KF agar
Nalidixic acid
2,3,5-Triphenyltetrazolium chloride
Ethanol
Potassium phosphate
Sodium hydroxide
Sodium carbonate
Deionized water

2.0 Media and Diluent Preparation

2.1 Enrichment Media Preparation: Single Strength Azide Dextrose Broth

2.1.1 Weigh 34.7 g of azide dextrose broth into a 2 liter Erlenmeyer flask.
2.1.2 Add 1.0 liter of deionized water and bring to a boil with continuous stirring.
2.1.3 Dispense the medium in 10.0 ml aliquots into 16 x 150 mm test tubes.
2.1.4 Cap the test tubes and autoclave at 116°C for 10 minutes.
2.1.5 Store the media at 4°C in the dark for up to one month.

2.2 Fecal Streptococci Confirmation Media Preparation: EVA Broth

2.2.1 Weigh 35.8 g of EVA broth into a 2 liter Erlenmeyer flask.
2.2.2 Add 1.0 liter of deionized water and bring to a boil with continuous stirring.
2.2.3 Dispense the medium in 10.0 ml aliquots into 16 x 150 mm test tubes.
2.2.4 Cap the test tubes and autoclave at 121°C for 15 minutes.
2.2.5 Store the media at 4°C in the dark for up to one month.

2.0 Media and Diluent Preparation (continued)

2.3 Fecal Streptococci Confirmation Media Preparation: mE Agar

- 2.3.1 Weigh 71.4 g of mE agar powder into a 2 liter Erlenmeyer flask. Add 1.0 liter of deionized water and mix well.
- 2.3.2 Heat with continuous mixing. Boil for approximately 1 minute to completely dissolve the powder.
- 2.3.3 Autoclave at 121°C for 15 minutes.
- 2.3.4 Temper the media to 44-46°C for approximately 30 minutes.
- 2.3.5 Aseptically add:
0.24 g of nalidixic acid dissolved in 0.3 ml of sterile deionized water and 0.2 ml of 10N sodium hydroxide and 15.0 ml of sterile 1% 2,3,5 triphenyltetrazolium chloride.
- 2.3.6 Adjust the pH to 7.1 \pm 0.01.
- 2.3.7 Dispense the medium into 9 x 50 mm petri dishes in 4.5 ml aliquots and allow to solidify.
- 2.3.8 Store mE agar plates inverted at 4°C in the dark for up to one month.

2.4 Enterococci Confirmation Media Preparation: KF Agar

- 2.4.1 Weigh 76.4 g of KF agar into 2 liter Erlenmeyer flask add 1.0 liter of deionized water and mix well.
- 2.4.2 Bring the medium to a boil and continue to heat for 5 minutes, no longer.
- 2.4.3 Temper the media to 44-46°C for approximately 30 minutes.
- 2.4.4 Aseptically add 1.0 ml of a 1% solution of 2,3,5 triphenyltetrazolium chloride.
- 2.4.5 If necessary, adjust the pH to 7.2 \pm 0.2 with 10% sodium carbonate.
- 2.4.6 Dispense the medium into 9 x 50 mm petri dishes in 4.5 ml aliquots.
- 2.4.7 Store KF agar plates inverted at 4°C in the dark for up to one month.

2.5 Fecal Streptococci Confirmation Media Preparation: Esculin Iron Agar

- 2.5.1 Weigh 16.5 g of EIA powder into a 2 liter Erlenmeyer flask. Add 1.0 liter of deionized water and mix well.
- 2.5.2 Heat with continuous mixing. Boil for approximately 1 minute to completely dissolve the powder.
- 2.5.3 Autoclave at 121°C for 15 minutes.
- 2.5.4 Temper the media to 44-46°C for approximately 30 minutes.
- 2.5.5 Dispense the medium into 9 x 50 mm petri dishes in 4.5 ml aliquots and allow to solidify.
- 2.5.6 Store EIA plates inverted at 4°C in the dark for up to one month.

2.0 Media and Diluent Preparation (continued)

2.6 Stock Phosphate Buffer Solution: Buffered Dilution Water

- 2.6.1 In a 1 liter volumetric flask, dissolve 34.0 g of potassium phosphate in 500 ml of deionized water.
- 2.6.2 Adjust to pH 7.2 with 10N sodium hydroxide.
- 2.6.3 Make up to 1 liter with deionized water.
- 2.6.4 Add the buffer solution to a 1 liter polypropylene bottle and autoclave at 121°C for 15 minutes.
- 2.6.5 Store at 4°C in the dark for up to one month.

2.7 Diluent Preparation: Phosphate Buffered Dilution Water

- 2.7.1 Add 20 liters of deionized water to an autoclavable carboy.
- 2.7.2 Add 25 ml of stock phosphate buffer solution.
- 2.7.3 Autoclave at 121°C for 45 minutes.
- 2.7.4 Store at room temperature for up to two weeks.

2.8 Diluent Preparation: Phosphate Buffered Dilution Blanks

- 2.8.1 Add 1.0 liter of deionized water to a 2 liter Erlenmeyer flask.
- 2.8.2 Add 1.25 ml of stock phosphate buffer solution.
- 2.8.3 Dispense the medium in 9.2 ml aliquots into 16 x 150 mm test tubes, 81.0 ml aliquots into 100 ml graduated dilution bottles and 100.0 ml aliquots into 100 ml graduated dilution bottles.
- 2.8.4 Cap the test tubes and/or dilution bottles and autoclave at 121°C for 15 minutes.
- 2.8.5 Refrigerate at 4°C in the dark for up to one month. **NOTE: Always check the volume of dilution water present in dilution blanks with each use. Discard any batch of buffered dilution water where evaporation has resulted in an inappropriate volume of buffered dilution water.**

3.0 Sample Storage

3.1 Shellfish Storage Containers

- 3.1.1 Use containers for shell-stock which are waterproof and durable enough to withstand the cutting action of the shellfish and abrasion during transport. Waterproof paper bags, paraffined cardboard cups or plastic bags are suitable containers.
- 3.1.2 Use sterile wide-mouth jars with watertight closure for freshly shucked shellfish samples. Transfer samples of the final product to the sample jar with sterile forceps or spoon.
- 3.1.3 Use one or two packages of frozen shucked shellfish, containing 10 to 12 animals each, as one sample. Transfer core samples taken from larger blocks to sterile wide-mouth jars for transportation to the laboratory.

3.0 Sample Storage (continued)

3.2 Shellfish Storage Conditions and Temperature

- 3.2.1 Keep shell-stock samples in dry storage at a temperature above freezing but lower than 10°C until examination. Do not allow samples to come in contact with ice.
- 3.2.2 Refrigerate samples of shucked shellfish immediately after collection by packing in crushed ice until examination.
- 3.2.3 Keep samples of frozen shucked shellfish in the frozen state or at temperatures close to those at which the commercial stock was maintained. When this is not possible, pack samples of frozen shucked shellfish in crushed ice until examination.

3.3 Shellfish Identification Records

- 3.3.1 Maintain records of identification for each shellfish sample to enter the laboratory. Include the date, time, and place of collection, area the shellfish were harvested, the date and time of harvesting and the physical and chemical measurements of the harvesting water (if applicable). Also include the storage conditions between harvesting and collection.
- 3.3.2 Mark individual containers of shellfish samples for identification. Also, include identification information on a descriptive form to accompany the sample.
- 3.3.3 Record the time elapsed between collection and examination for each shellfish sample examined in the laboratory. Examine samples of shell stock and shucked, unfrozen shellfish within 6 hours after collection and in no case examine shellfish if they have been held more than 24 hours after collection.
- 3.3.4 Record the identification of the shipper, the date of shipment, and the harvesting area as well as the date, time and place of collection for shellfish samples collected in market areas and when other specified information is not available.

4.0 Sample Preparation

4.1 Shellfish in the Shell - Cleaning the Shells

- 4.1.1 Use a minimum of 12 shellfish in order to obtain a representative sample (approximately 200 g of shellfish liquor and meats) and to allow for the selection of sound animals suitable for shucking. Collect at least 200 g of sample when examining shucked or frozen shellfish.
- 4.1.2 Scrub hands thoroughly with soap and water.
- 4.1.3 Scrape off all growth and loose material from the shell. Scrub the shell stock with a sterile stiff brush under running water of drinking-water quality, paying particular attention to the crevices of the shells.

4.0 Sample Preparation (continued)

4.1 Shellfish in the Shell - Cleaning the Shells

4.1.4 Place the cleaned shell stock in clean containers or on clean towels to drain in the air.

4.2 Removal of Shell Contents

4.2.1 Before starting the removal of shell contents, scrub hands thoroughly with soap and water and rinse with 70% alcohol.

4.2.2 **OYSTERS:** Hold the oyster in the hand or on a fresh clean paper towel on the bench with the deep shell on the bottom. Using a sterile oyster knife, insert the point between the shells on the ventral side (at the right when the hinge is pointed away from the examiner), about 1/4 the distance from the hinge to the bill. Entry may also be made at the bill. Cut the adductor muscle from the upper flat shell and pry the shell wide enough to drain the shell liquor into a sterile tared blender. The upper shell may then be pried loose at the hinge, discarded, and the meats transferred to a sterile tared blender after severing the muscle attachment to the lower shell.

4.2.3 **HARD CLAMS:** Enter the hard clam with a sterile, thin-bladed knife similar to a paring knife. To open the clam, hold it in the hand, place the edge of the knife at the junction of the bills, and force it between the shells with a squeezing motion. An alternative method is to nibble a small hole in the bill, and with the knife, sever the two adductor muscles. Drain the shell liquor into a sterile tared blender. Cut the adductor muscles from the shells and transfer the body of the animal to the blender.

4.2.4 **OTHER CLAMS:** Shuck soft clams, butter clams, surf clams and similar species with a sterile paring knife, entering at the siphon end and cutting the adductor muscles first from the top valve and then from the bottom valve. Drain the shell liquor into a sterile tared blender. Transfer the body of the animal to the blender.

4.2.5 **MUSSELS:** Remove the byssal threads during the cleansing of the shell. Enter the mussel at the byssal opening. Insert the knife and spread the shells apart with a twisting motion, draining the liquor of the shellfish into a sterile tared blender. Transfer the body of the animal to the blender.

4.3 Dilution and Grinding

4.3.1 Weigh the shellfish meats and liquors.

4.3.2 Add an equal amount, by weight, of phosphate buffered dilution water to the blender.

4.3 Dilution and Grinding (continued)

4.3.3 Grind for 60-120 sec in a sterile waring blender operating at approximately 14,000 RPM. Avoid excessive grinding to prevent overheating.

5.0 Sample Analyses

5.1 Presumptive Test Using Azide Dextrose Broth

5.1.1 Inoculate each of 5 single strength azide dextrose broth tubes with 2.0 ml of the prepared sample (equal to 1.0 g of shellfish), 5 single strength azide dextrose broth tubes with 1.0 ml of a 10 fold dilution of the prepared sample (equal to 0.1 g of shellfish) and 5 single strength azide dextrose broth tubes with 1.0 ml of a 100 fold dilution of the prepared sample (equal to 0.01 g of shellfish).

5.1.2 In order to avoid indeterminate results, perform extensions of the dilutions mentioned.

5.1.3 Incubate tubes at $35.0 \pm 0.5^\circ\text{C}$ for 24 ± 2 hours.

5.1.4 Examine tubes for the presence/absence of turbidity.

5.1.5 Score tubes with turbidity and perform confirmation tests for only those tubes with turbidity at this time.

5.1.6 Return tubes to $35.0 \pm 0.5^\circ\text{C}$ for an additional 24 ± 2 hours.

5.1.7 Re-examine the tubes at 48 ± 3 hours for the presence/absence of turbidity.

5.1.8 Score tubes with turbidity and perform confirmation tests for only those tubes with turbidity at this time.

5.1.9 Absence of turbidity at the end of 48 ± 3 hours constitutes a negative test result for the fecal streptococci and enterococci groups.

5.2 Fecal Streptococci Confirmation Test Using EVA Broth

5.2.1 Mix the presumptive azide dextrose broth tubes by gentle shaking or rotating.

5.2.2 Transfer 1.0 ml of sample using sterile pipets from azide dextrose broth tubes with turbidity/growth to EVA Broth tubes.

5.2.3 Incubate tubes at $35.0 \pm 0.5^\circ\text{C}$ for 48 ± 3 hours.

5.2.4 Examine tubes for the presence/absence of a purple button and/or turbidity.

5.2.5 Score tubes with a purple button and/or turbidity positive for the fecal streptococci group.

5.2.6 Score tubes with no purple button and/or turbidity negative for the fecal streptococci group.

5.0 Sample Analyses (continued)

5.3 Fecal Streptococci Confirmation Test Using KF Agar

- 5.3.1 Mix the presumptive azide dextrose broth tubes by gentle shaking or rotating.
- 5.3.2 Place a sterile 0.45 micron membrane on a KF agar plate.
- 5.3.3 Transfer sample using sterile transfer sticks from azide dextrose broth tubes with turbidity to the membrane of a KF plate.
- 5.3.4 Repeat this process for each tube with turbidity. Use one plate for each dilution (maximum 5 transfers/ plate).
- 5.3.5 Incubate the inverted plates at $35.0 \pm 0.5^\circ\text{C}$ for 48 hours.
- 5.3.6 Score carmine or red transfers positive for the fecal streptococci group.

5.4 Enterococci Confirmation Test Using mE and EIA Agar

- 5.4.1 Mix the presumptive azide dextrose broth tubes by gentle shaking or rotating.
- 5.4.2 Place a sterile 0.45 micron membrane on a mE agar plate.
- 5.4.3 Transfer sample using sterile transfer sticks from azide dextrose broth tubes with turbidity to the membrane of a mE plate.
- 5.4.4 Repeat this process for each tube with turbidity. Use one plate for each dilution (maximum 5 transfers/ plate).
- 5.4.5 Incubate the inverted plates at $41.0 \pm 0.5^\circ\text{C}$ for 48 hours.
- 5.4.6 After the incubation period, score all typical, presumptive enterococci transfers (pink to red in color).
- 5.4.7 Transfer membranes to EIA plates and incubate at $41.0 \pm 0.5^\circ\text{C}$ for 20 minutes.
- 5.4.8 Score transfers which possess a brown/black precipitate positive for the enterococci group.

6.0 Using the Most Probable Number Geometric Series

- 6.1 Count the number of positive tubes from each of the three dilutions performed.
- 6.2 Use the number of positive tubes from each dilution to form a 3 digit code.
- 6.3 Locate your three digit code on the most probable number geometric series chart.
- 6.4 Read the MPN number which corresponds to your three digit code. This is your density per 100 g of sample.
- 6.5 If, instead of 10, 1.0, and 0.1 ml a combination of portions of 100, 10, and 1 ml is used, record the MPN as 0.1 times the figure in the tabulation. On the other hand, should a combination of corresponding portions of 1.0, 0.1 and 0.01 ml be planted, record 10 times the figure in the tabulation; should a combination of portions of 0.1, 0.01, and 0.001 ml be planted, record 100 times the figure in the tabulation.

6.0 Using the Most Probable Number Geometric Series (continued)

6.6 When more than 3 dilutions are employed in a decimal series of dilutions, the results from only 3 of them are significant. To select the 3 dilutions to be used in determining the MPN index, using the system of 5 tubes of each dilutions as an example, choose the highest dilution which gives positive results in all of the 5 portions tested (no lower dilution giving any negative results) and the 2 next succeeding high dilutions. The results of these 3 volumes should then be used in the computations of the MPN index. In examples given below, significant dilutions results are shown in boldface.

The number in the numerator represents positive tubes; that in the denominator, the total tubes planted.

	1 ml or g	0.1 ml or g	0.01 ml or g	0.001 ml or g
(a)	5/5	5/5	2/5	0/5
(b)	5/5	4/5	2/5	0/5
(c)	0/5	1/5	0/5	0/5
(d)	5/5	3/5	1/5	1/5
(e)	5/5	3/5	2/5	0/5

In example (c) above, the first 3 dilutions are taken in order to throw the positive result into the middle dilution. When a case arises such as is shown in (d), where a positive occurs in a dilution higher than the 3 chosen according to the rule, it is included in the result of the highest chosen dilution (e).

7.0 Trouble Shooting

- 7.1 Thoroughly clean the shells of each shellfish, especially the hinge regions.
- 7.2 Adequately mix samples and dilutions of samples prior to addition to enrichment tubes.
- 7.3 Adequately mix presumptive enrichment tubes prior to addition of sample to confirmation enrichment tubes and media.

8.0 Statistical Analysis and Data Usage

- 8.1 Tabulate and summarize the data.
- 8.2 Use data in conjunction with other indicator tests to determine sanitary quality.

9.0 References

- 9.1 American Public Health Association, Inc. 1970. Recommended Procedures for the Examination of Sea Water and Shellfish. Washington, DC. p. 1-105.

Environmental Testing Center
Standard Operating Procedure
MPN Shellfish
Fecal Streptococci and Enterococci
October 1994

9.0 **References (continued)**

- 9.2 American Public Health Association, American Water Works Association, Water Pollution Control Federation. 1989. Standard Methods for the Examination of Water and Wastewater, Seventeenth Edition. Washington, DC. Chapter 9.
- 9.3 Levin, M.A., S.R. Fischer, V.J. Cabelli. 1975. Membrane Filter Technique for Enumeration of Enterococci in Marine Waters. *Appl. Microbiol.* 30:66-71.

MOST PROBABLE NUMBER METHOD FOR THE ENUMERATION OF FECAL STREPTOCOCCI AND ENTEROCOCCI IN SEDIMENT

Point of Contact

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The term "fecal streptococci" refers to the Lancefield Group D bacteria. The term "enterococci" refers to a specific subgroup of fecal streptococci including *Streptococcus faecalis*, *Streptococcus faecium*, *Streptococcus avium* and *Streptococcus gallinarum*. Fecal streptococci and enterococci are found in the intestines of humans and other animals, where they behave as commensals of limited pathogenic potential. Because these organisms are associated with fecal wastes of man and animals, they can be isolated from contaminated waters containing such waste. Enumeration of fecal streptococci and enterococci *sp.* using the most probable number method is a means of determining the sanitary quality of sediment.

1.0 Necessary Materials and Equipment

1.1 Glassware, Plasticware, Equipment and Disposable Supplies

- 2 liter Erlenmeyer flasks
- 16 x 150 mm test tubes
- 20 x 150 mm test tubes
- 16 and 20 mm test tube caps
- 16 and 20 mm test tube racks
- 1.0 and 10.0 ml sterile pipettes
- 20 liter polypropylene carboy
- Heating mantle with magnetic stirrer and stir bars
- Balance and weigh boats
- Autoclave
- Cornwall syringe
- Air incubators $35.0 \pm 0.5^{\circ}\text{C}$
- Air incubator $41.0 \pm 0.5^{\circ}\text{C}$
- Waterbath $44-46^{\circ}\text{C}$
- Sterile specimen cup
- Timer
- Sterile transfer sticks
- pH meter

1.0 Necessary Materials and Equipment (continued)

1.1 Glassware, Plasticware, Equipment and Disposable Supplies

Alcohol burner
Membranes, 0.45 micron
Membrane forceps

1.2 Media and Diluent

Azide dextrose broth
Ethyl Violet Azide (EVA) Broth
mE agar
Esculin iron agar
KF agar
Nalidixic acid
2,3,5-Triphenyltetrazolium chloride
Ethanol
Potassium phosphate
Sodium hydroxide
Sodium carbonate
Deionized water

2.0 Media and Diluent Preparation

2.1 Enrichment Media Preparation: Double Strength Azide Dextrose Broth

2.1.1 Weigh 69.4 g of azide dextrose broth into a 2 liter Erlenmeyer flask.
2.1.2 Add 1.0 liter of deionized water and bring to a boil with continuous stirring.
2.1.3 Dispense the medium in 10.0 ml aliquots into 16 x 150 mm test tubes.
2.1.4 Cap the test tubes and autoclave at 116°C for 10 minutes.
2.1.5 Store the media at 4°C in the dark for up to one month.

2.2 Enrichment Media Preparation: Single Strength Azide Dextrose Broth

2.2.1 Weigh 34.7 g of azide dextrose broth into a 2 liter Erlenmeyer flask.
2.2.2 Add 1.0 liter of deionized water and bring to a boil with continuous stirring.
2.2.3 Dispense the medium in 10.0 ml aliquots into 16 x 150 mm test tubes.
2.2.4 Cap the test tubes and autoclave at 116°C for 10 minutes.
2.2.5 Store the media at 4°C in the dark for up to one month.

2.3 Fecal Streptococci Confirmation Media Preparation: EVA Broth

2.3.1 Weigh 35.8 g of EVA broth into a 2 liter Erlenmeyer flask.
2.3.2 Add 1.0 liter of deionized water and bring to a boil with continuous stirring.
2.3.3 Dispense the medium in 10.0 ml aliquots into 16 x 150 mm test tubes.
2.3.4 Cap the test tubes and autoclave at 121°C for 15 minutes.

2.0 Media and Diluent Preparation (continued)

2.3.5 Store the media at 4°C in the dark for up to one month.

2.4 Fecal Streptococci Confirmation Media Preparation: mE Agar

2.4.1 Weigh 71.4 g of mE agar powder into a 2 liter Erlenmeyer flask. Add 1.0 liter of deionized water and mix well.

2.4.2 Heat with continuous mixing. Boil for approximately 1 minute to completely dissolve the powder.

2.4.3 Autoclave at 121°C for 15 minutes.

2.4.4 Temper the media to 44-46°C for approximately 30 minutes.

2.4.5 Aseptically add:

0.24 g of nalidixic acid dissolved in 0.3 ml of sterile deionized water and 0.2 ml of 10N sodium hydroxide and 15.0 ml of sterile 1% 2,3,5 triphenyltetrazolium chloride.

2.4.6 Adjust the pH to 7.1 ± 0.01 .

2.4.7 Dispense the medium into 9 x 50 mm petri dishes in 4.5 ml aliquots and allow to solidify.

2.4.8 Store mE agar plates inverted at 4°C in the dark for up to one month.

2.5 Enterococci Confirmation Media Preparation: KF Agar

2.5.1 Weigh 76.4 g of KF agar into 2 liter Erlenmeyer flask add 1.0 liter of deionized water and mix well.

2.5.2 Bring the medium to a boil and continue to heat for 5 minutes, no longer.

2.5.3 Temper the media to 44-46°C for approximately 30 minutes.

2.5.4 Aseptically add 1.0 ml of a 1% solution of 2,3,5 triphenyltetrazolium chloride.

2.5.5 If necessary, adjust the pH to 7.2 ± 0.2 with 10% sodium carbonate.

2.5.6 Dispense the medium into 9 x 50 mm petri dishes in 4.5 ml aliquots.

2.5.7 Store KF agar plates inverted at 4°C in the dark for up to one month.

2.6 Fecal Streptococci Confirmation Media Preparation: Esculin Iron Agar

2.6.1 Weigh 16.5 g of EIA powder into a 2 liter Erlenmeyer flask. Add 1.0 liter of deionized water and mix well.

2.6.2 Heat with continuous mixing. Boil for approximately 1 minute to completely dissolve the powder.

2.6.3 Autoclave at 121°C for 15 minutes.

2.6.4 Temper the media to 44-46°C for approximately 30 minutes.

2.6.5 Dispense the medium into 9 x 50 mm petri dishes in 4.5 ml aliquots and allow to solidify.

2.6.6 Store EIA plates inverted at 4°C in the dark for up to one month.

2.0 Media and Diluent Preparation (continued)

2.7 Stock Phosphate Buffer Solution: Buffered Dilution Water

- 2.7.1 In a 1 liter volumetric flask, dissolve 34.0 g of potassium phosphate in 500 ml of deionized water.
- 2.7.2 Adjust to pH 7.2 with 10N sodium hydroxide.
- 2.7.3 Make up to 1 liter with deionized water.
- 2.7.4 Add the buffer solution to a 1 liter polypropylene bottle and autoclave at 121°C for 15 minutes.
- 2.7.5 Store at 4°C in the dark for up to one month.

2.8 Diluent Preparation: Phosphate Buffered Dilution Water

- 2.8.1 Add 20 liters of deionized water to an autoclavable carboy.
- 2.8.2 Add 25 ml of stock phosphate buffer solution.
- 2.8.3 Autoclave at 121°C for 45 minutes.
- 2.8.4 Store at room temperature for up to two weeks.

2.9 Diluent Preparation: Phosphate Buffered Dilution Blanks

- 2.9.1 Add 1.0 liter of deionized water to a 2 liter Erlenmeyer flask.
- 2.9.2 Add 1.25 ml of stock phosphate buffer solution.
- 2.9.3 Dispense the medium in 9.2 ml aliquots into 16 x 150 mm test tubes, and 90.0 ml aliquots into 100 ml graduated dilution bottles.
- 2.9.4 Cap the test tubes and/or dilution bottles and autoclave at 121°C for 15 minutes.
- 2.9.5 Refrigerate at 4°C in the dark for up to one month. **NOTE: Always check the volume of dilution water present in dilution blanks with each use. Discard any batch of buffered dilution water where evaporation has resulted in an inappropriate volume of buffered dilution water.**

3.0 Sample Storage

3.1 Sediment Storage Containers

- 3.1.1 Use containers for sediments which are waterproof and durable enough to withstand the cutting action of the sediment particles and abrasion during transport. Waterproof paper bags, paraffined cardboard cups or plastic bags are suitable containers.

3.2 Sediment Storage Conditions and Temperature

- 3.2.1 Keep sediment samples in dry storage at a temperature above freezing but lower than 10°C until examination. Do not allow samples to come in contact with ice.

3.0 Sample Storage (continued)

3.3 Sediment Identification Records

- 3.3.1 Maintain records of identification for each sediment sample to enter the laboratory. Include the date, time, and location of collection, and the physical and chemical measurements of the environment (if applicable).
- 3.3.2 Mark individual containers of sediment samples for identification. Also, include identification information on a descriptive form to accompany the sample.
- 3.3.3 Record the time elapsed between collection and examination for each sediment sample examined in the laboratory. Examine samples within 6 hours after collection and in no case examine sediments if they have been held more than 24 hours after collection.

4.0 Sample Preparation

- 4.1 Use a sterile tongue depressor to homogenize sediment samples in their respective collection containers.
- 4.2 Note the consistency of the sediment (mud, sand, rocks) on the data sheet.
- 4.3 Weigh 20 g of the homogenized sediment sample into a sterile specimen cup.
- 4.4 Add 180 ml of sterile phosphate buffered dilution water to the cup.
- 4.5 Alcohol and flame a magnetic stir bar before adding it to the sediment and buffer mixture.
- 4.6 Mix the sediment and buffer for 2 minutes prior to transfer to media or dilution blanks.

5.0 SAMPLE ANALYSES

5.1 Presumptive Test Using Azide Dextrose Broth

- 5.1.1 Inoculate each of 5 double strength azide dextrose broth tubes with 2.0 ml of the prepared sample (equal to 1.0 g of sediment), 5 single strength azide dextrose broth tubes with 1.0 ml of a 10 fold dilution of the prepared sample (equal to 0.1 g of sediment) and 5 single strength azide dextrose broth tubes with 1.0 ml of a 100 fold dilution of the prepared sample (equal to 0.01 g of sediment).
- 5.1.2 In order to avoid indeterminate results, perform extensions of the dilutions mentioned.
- 5.1.3 Incubate tubes at $35.0 \pm 0.5^\circ\text{C}$ for 24 ± 2 hours.
- 5.1.4 Examine tubes for the presence/absence of turbidity.
- 5.1.5 Score tubes with turbidity and perform confirmation tests for only those tubes with turbidity at this time.
- 5.1.6 Return tubes to $35.0 \pm 0.5^\circ\text{C}$ for an additional 24 ± 2 hours.

5.0 Sample Analyses (continued)

- 5.1.7 Re-examine the tubes at 48 ± 3 hours for the presence/absence of turbidity.
- 5.1.8 Score tubes with turbidity and perform confirmation tests for only those tubes with turbidity at this time.
- 5.1.9 Absence of turbidity at the end of 48 ± 3 hours constitutes a negative test result for the fecal streptococci and enterococci groups.
- 5.2 Fecal Streptococci Confirmation Test Using EVA Broth**
 - 5.2.1 Mix the presumptive azide dextrose broth tubes by gentle shaking or rotating.
 - 5.2.2 Transfer 1.0 ml of sample using sterile pipets from azide dextrose broth tubes with turbidity/growth to EVA Broth tubes.
 - 5.2.3 Incubate tubes at $35.0 \pm 0.5^\circ\text{C}$ for 48 ± 3 hours.
 - 5.2.4 Examine tubes for the presence/absence of a purple button and/or turbidity.
 - 5.2.5 Score tubes with a purple button and/or turbidity positive for the fecal streptococci group.
 - 5.2.6 Score tubes with no purple button and/or turbidity negative for the fecal streptococci group.
- 5.3 Fecal Streptococci Confirmation Test Using KF Agar**
 - 5.3.1 Mix the presumptive azide dextrose broth tubes by gentle shaking or rotating.
 - 5.3.2 Place a sterile 0.45 micron membrane on a KF agar plate.
 - 5.3.3 Transfer sample using sterile transfer sticks from azide dextrose broth tubes with turbidity to the membrane of a KF plate.
 - 5.3.4 Repeat this process for each tube with turbidity. Use one plate for each dilution (maximum 5 transfers/ plate).
 - 5.3.5 Incubate the inverted plates at $35.0 \pm 0.5^\circ\text{C}$ for 48 hours.
 - 5.3.6 Score carmine or red transfers positive for the fecal streptococci group.
- 5.4 Enterococci Confirmation Test Using mE and EIA Agar**
 - 5.4.1 Mix the presumptive azide dextrose broth tubes by gentle shaking or rotating.
 - 5.4.2 Place a sterile 0.45 micron membrane on a mE agar plate.
 - 5.4.3 Transfer sample using sterile transfer sticks from azide dextrose broth tubes with turbidity to the membrane of a mE plate.
 - 5.4.4 Repeat this process for each tube with turbidity. Use one plate for each dilution (maximum 5 transfers/ plate).
 - 5.4.5 Incubate the inverted plates at $41.0 \pm 0.5^\circ\text{C}$ for 48 hours.

5.0 Sample Analyses (continued)

- 5.4.6 After the incubation period, score all typical, presumptive enterococci transfers (pink to red in color).
- 5.4.7 Transfer membranes to EIA plates and incubate at $41.0 \pm 0.5^\circ\text{C}$ for 20 minutes.
- 5.4.8 Score transfers which possess a brown/black precipitate positive for the enterococci group.

6.0 Using the Most Probable Number Geometric Series

- 6.1 Count the number of positive tubes from each of the three dilutions performed.
- 6.2 Use the number of positive tubes from each dilution to form a 3 digit code.
- 6.3 Locate your three digit code on the most probable number geometric series chart.
- 6.4 Read the MPN number which corresponds to your three digit code. This is your density per 100 g of sample.
- 6.5 If, instead of 10, 1.0, and 0.1 ml a combination of portions of 100, 10, and 1 ml is used, record the MPN as 0.1 times the figure in the tabulation. On the other hand, should a combination of corresponding portions of 1.0, 0.1 and 0.01 ml be planted, record 10 times the figure in the tabulation; should a combination of portions of 0.1, 0.01, and 0.001 ml be planted, record 100 times the figure in the tabulation.
- 6.6 When more than 3 dilutions are employed in a decimal series of dilutions, the results from only 3 of them are significant. To select the 3 dilutions to be used in determining the MPN index, using the system of 5 tubes of each dilutions as an example, choose the highest dilution which gives positive results in all of the 5 portions tested (no lower dilution giving any negative results) and the 2 next succeeding high dilutions. The results of these 3 volumes should then be used in the computations of the MPN index. In examples given below, significant dilutions results are shown in boldface.

The number in the numerator represents positive tubes; that in the denominator, the total tubes planted.

	1 ml or g	0.1 ml or g	0.01 ml or g	0.001 ml or g
(a)	5/5	5/5	2/5	0/5
(b)	5/5	4/5	2/5	0/5
(c)	0/5	1/5	0/5	0/5
(d)	5/5	3/5	1/5	1/5
(e)	5/5	3/5	2/5	0/5

6.0 Using the Most Probable Number Geometric Series (continued)

In example (c) above, the first 3 dilutions are taken in order to throw the positive result into the middle dilution. When a case arises such as is shown in (d), where a positive occurs in a dilution higher than the 3 chosen according to the rule, it is included in the result of the highest chosen dilution (e).

7.0 Trouble Shooting

- 7.1 Thoroughly homogenize sediment samples before preparation of dilutions.
- 7.2 Adequately mix samples and dilutions of samples prior to addition to enrichment tubes.
- 7.3 Adequately mix presumptive enrichment tubes prior to addition of sample to confirmation enrichment tubes and media.

8.0 Statistical Analysis and Data Usage

- 8.1 Tabulate and summarize the data.
- 8.2 Use data in conjunction with other indicator tests to determine sanitary quality.

9.0 References

- 9.1 American Public Health Association, Inc. 1970. Recommended Procedures for the Examination of Sea Water and Shellfish. Washington, DC. p. 1-105.
- 9.2 American Public Health Association, American Water Works Association, Water Pollution Control Federation. 1989. Standard Methods for the Examination of Water and Wastewater, Seventeenth Edition. Washington, DC. Chapter 9.
- 9.3 Levin, M.A., S.R. Fischer, V.J. Cabelli. 1975. Membrane Filter Technique for Enumeration of Enterococci in Marine Waters. Appl. Microbiol. 30:66-71.

POINT OF CONTACT:

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I. OBJECTIVE

Deployment and retrieval of caged bivalves for environmental monitoring.

II. NECESSARY MATERIALS AND EQUIPMENT

- 17' Whaler or larger vessel
 - Coolers for holding the organisms during transport
 - Twine
 - Pocket knife
 - Foul weather gear
 - First Aid Kit
 - Mussel trawls (per trawl, see schematic):
 - 4 cinder blocks
 - 4 trawl floats
 - 4 mussel baskets
 - 2 lobster trap buoys
 - 2 small (1lb) weights
 - 1 trawl line, 1/2 - 3/4 inch polypropylene, approximate length = 2 x maximum depth + 20m.
- NOTE--There are no hard and fast rules regarding the length of the trawl line, flexibility permits adaptation to the various constraints encountered at any particular deployment location. One should make certain that under no circumstances will excess line be found floating at or near the surface.
- 4 leaders, 2-3m lengths of the same line as the trawl line
 - 4 plastic ties
 - 4 2-3m lengths of "tire cord" (small diameter nylon line)

III. METHODS

In order to facilitate transport and deployment, it is recommended that trawl lines be partially assembled before going into the field. Prepare the trawl line but do not attach cinder blocks, trawl floats, or mussel baskets until just prior to deployment.

A. Trawl line construction

At the laboratory/on land:

1. After consulting nautical charts, cut the desired length of line (minimum of 2 x the high water depth + 20m).
2. Attach one buoy to each end, one or two overhand knots above and below the buoy should be sufficient. A loop of some sort above the buoy will facilitate retrieval with a boat hook.
3. Attach the two small weights to the main trawl line 2-3m below the two buoys--this prevents line from floating at the surface during low tide.
4. Attach (by knotting or splicing) the 4 leaders to the main trawl line. Attach the first at the maximum total depth + 2m, and the rest at consecutive 5m intervals.
5. Coil trawl for transport to the field. Securing the coil with twine can make a bulky trawl more manageable.
6. Attach one 2-3m section of "small" nylon line (tire cord) to each of the trawl floats. Make certain that the length of line is appropriate for the trawl floats be suspended 1m above the substrate. Tie them together in sets of four for transport.

In the field:

7. Stand 4 cinder blocks upright in a line position near the point from which they will be deployed.
8. Uncoil the trawl line and attach one cinder block to the end of each of the leaders using a clove hitch. The clove hitch can be secured with either a half hitch or by twisting open the braid of the line above the clove hitch and passing the free end of the line through the braid.

9. Secure the trawl floats to the trawl line just above the knot at each cement block, again use a clove hitch. The trawl float should float 1m above the substrate when deployed.

10. Using the plastic ties, attach the mussel basket to the trawl float.

B. Deployment

1. Deploy the trawl sequentially beginning with one buoy then each of the cinder blocks and finally the second buoy. Be deliberate. Lower each block into the water, making certain that the trawl float and the mussel basket float free, and slowly pay out the line by hand until reaching the next block. Repeat the procedure for each block. Avoid dropping the blocks on top of one another by maneuvering the boat in low speed or by using the prevailing wind to stretch the trawl as it is deployed. Once deployed, it may be necessary to tow one end of the trawl in order to fully stretch the trawl.

C. Retrieval

1. This is essentially deployment in reverse. Pick up the "down wind" buoy.
2. Pull the trawl up one block at a time.
3. Once the entire trawl is on deck, disassemble placing the mussel baskets in coolers, piling the cinder blocks together, tying the trawl floats together, and coiling the trawl.
4. Retrieve the next trawl.

IV. TROUBLE SHOOTING/HINTS

1. Set trawls 90deg to the predominant currents when ever possible as this helps to minimize tangling.
2. Avoid setting the trawl in a channel, mooring area or other high use area.
3. Avoid deploying mussel trawls near lobster trawls--they may not be there when you return.

4. Notify the local Harbormaster and appropriate regulatory agencies prior to deployment.

5. It may be necessary to make adjustments to the trawls in the field; therefore, it is worthwhile to bring extra line, blocks, and buoys if space permits. For example, where there is not enough space to deploy an entire trawl, it may be feasible to deploy each block individually, thus requiring extra line and floats.

6. In high energy zones with hard bottoms it may be necessary to use heavier/denser weights than cinder blocks (attaching lengths of chain to the trawl line between the cinder blocks may be sufficient). Whenever possible, use the wind, don't fight it.

V. STATISTICAL ANALYSIS AND DATA USAGE

Not applicable.

VI. REFERENCES

None.

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1. OBJECTIVE

The following document defines the standard operating procedures for the preparation of marine tissues, specifically for the American lobster, for organic and inorganic analysis.

2. SAFETY

Lobsters may be contaminated with hazardous biological or chemical constituents. Proper attire should be worn.

3. NECESSARY MATERIALS AND EQUIPMENT

Dissecting knife: stainless steel with replaceable blade

Teflon pad

Stainless steel scissors

Acid stripped capped jars (no aluminum foil) with teflon lined closures - sizes: 8 and 16 oz.

Lobster claw crusher

Teflon coated stainless steel forceps

4. METHODS

All dissection and removal of tissue from marine samples be conducted using good laboratory practices. All dissecting utensils and cutting surfaces should be free of contaminants prior to dissection.

- 4.1 Dissect and remove of tissue on a teflon or polyethylene cutting surface to minimize metal contamination.

- 4.2 Clean the cutting board and dissecting implements between treatments and composite reps with alconox soap, rinse with tap water 3x, rinse with distilled water 3x, and solvent rinsed with methanol.
- 4.3 Rinse the animal with seawater prior to dissection, making sure all particulate material and sediment has been removed.
- 4.4 Dissect the lobsters live using a stainless steel knife by my making an incision ventrally and equidistant from each side. The incision runs from the tip of the rostrum to the end of the tail.
- 4.5 Once this cut has been made, open the animal laterally to expose the organs for removal.
- 4.6 Remove the hepatopancreas first to prevent rupturing of the membrane. This organ is located on either side of the ventral mid-line in the posterior region of the carapace. Use stainless steel tweezers to remove this organ.
- 4.7 After the hepatopancreas has been removed, excise the muscle tissue from the tail using tweezers and spatula.
- 4.8 Break open the two claws of the lobster, ripper and crusher claw using a claw crusher and remove the tissue using the same instruments.
- 4.9. Place all tissue into acid stripped/muffled jars with teflon lined caps with the appropriate sample information. Lobster hepatopancreas can be placed into 8 oz. jars while lobster muscle tissue can placed into 16 oz. jars.
- 4.10 Samples should be placed into sealed zip lock bags, labeled appropriately, and stored.

*Note: Sample size for each tissue type should be at least 20-25g on a wet weight basis. Some tissues, such as the lobster hepatopancreas and flounder liver, will be less. A smaller sample size (15g) may be sufficient for these tissues. This amount of sample should provide sufficient material for both organic and inorganic analyses. Biomass considerations are important in determining the number of animals needed to collect. Sample size for lobsters will be contingent on the age class of the animal. Juvenile lobsters having carapace length between 4.0-4.5 cm., on average, contain 12.67g (wet weight) of muscle tissue and 2.32g (wet weight) hepatopancreas tissue per animal.

Larger size classes of lobster will contain proportionally more tissue on a wet weight basis.

5. REFERENCES

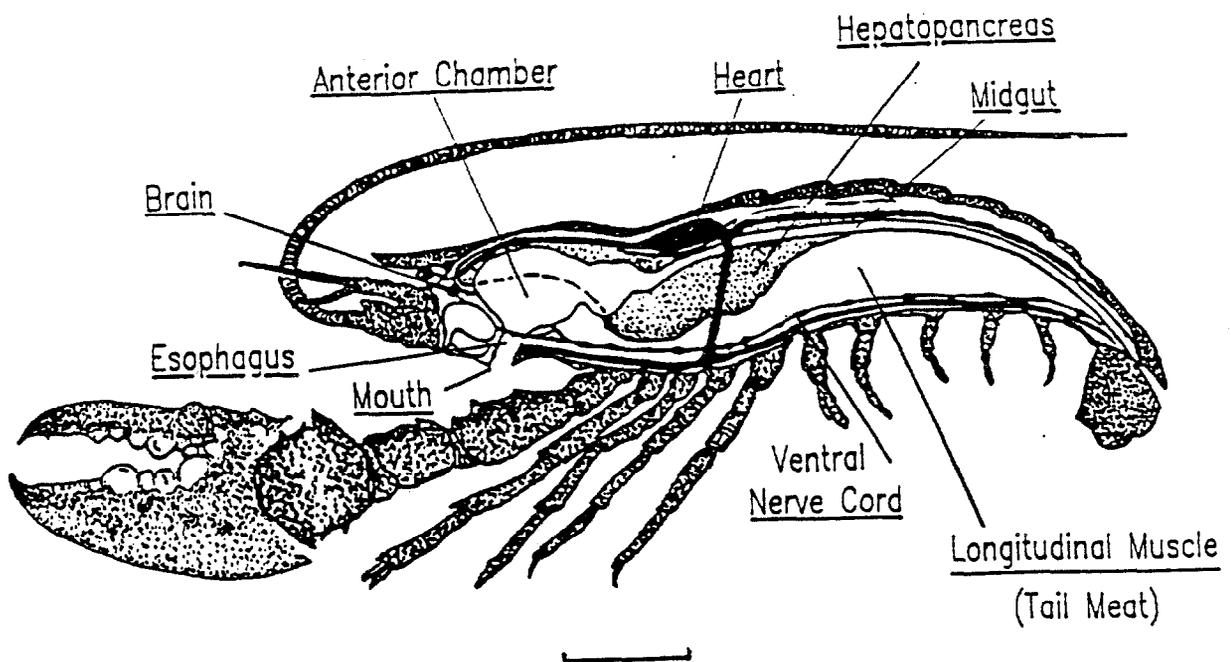
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AMERICAN LOBSTER

Internal Anatomy

(Sagittal view, showing major organs)



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1. OBJECTIVE

This document describes the procedures used to prepare elutriates from field collected sediment samples.

2. SAFETY

Sediment samples may contain hazardous biological or chemical constituents. Proper attire should be worn.

3. MATERIALS

Glassware
Detergent
Hydrochloric Acid Solution (10%)
Dilution water, use: disposal site water, clean seawater, or artificial sea/salt mixtures
Dredged material sample, at least 1 liter
Unfiltered dredging site water
Graduated cylinder
Magnetic stirrer
Stir bar
1ml pipette
Siphon
Receiving vessel
Centrifuge
Centrifuge tubes
Testing Chambers

4. METHODS

4.1 Cleaning the glassware

4.1.1 Wash with detergent.

4.1.2 Rinse five times with hot tap water.

- 4.1.3 Rinse with deionized water.
- 4.1.4 Place in a 10% HCL acid bath for at least 4 hours.
- 4.1.5 Remove from acid bath and rinse 4 times with deionized water.
- 4.2 Preparing the elutriate
 - 4.2.1 Subsample approximately 1 L of homogenized sample.
 - 4.2.2 Using volumetric displacement, combine, in a graduated cylinder, the homogenized sample with unfiltered dredging-site water in a sediment-to-water ratio of 1:4 on a volume basis.
 - 4.2.3 Place the sediment-water mixture and a stir bar into the labeled piece of glassware.
 - 4.2.4 Stir the mixture vigorously on a magnetic stirrer for 30 minutes.
 - 4.2.5 Hand stir the mixture every 10 minutes using the 1 ml pipette.
 - 4.2.6 At the end of the 30 minute mixing period, remove the mixture from the stirrer and allow to settle for 1 hour.
- 4.3 Preparing the supernatant
 - 4.3.1 Carefully siphon off the supernatant into the centrifugation vessels without disturbing the settled material.
 - 4.3.2 Centrifuge the supernatant until the suspension is clear enough at the first observation time for the organisms to be visible in the testing chambers.

NOTE: This step is only necessary with some very fine-grained dredged materials.
 - 4.3.3 Prepare 100%, 50% and 10% dilutions of the supernatant and use immediately for testing.

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1.0 OBJECTIVE

This Standard Operating Procedure (SOP) defines a method for determination of Sediment Oxygen Demand (SOD) and Biochemical Oxygen Demand (BOD) measurements for sediment and water-only samples collected at selected field stations. SOD and BOD are biochemical oxygen demands exerted by water and organic materials in bottom sediments and overlying waters.

The objective of this method is to estimate the SOD and BOD at the sediment water interface. The SOD of the sediment is determined from the difference between the oxygen demand of a sediment/water sample and the oxygen demand of a water only sample.

The primary goal of this SOP is to provide a consistent method for evaluating the sediment and water oxygen demand among selected field stations.

2.0 NECESSARY MATERIALS AND EQUIPMENT

- a. core tubes
- b. core caps
- c. core racks
- d. grab sampler
- e. DO bottles
- f. bulk water container
- g. aeration pump, air stone, tubing
- h. field DO sampling kit and/or meter, DO SOP
- i. sealing tape
- j. tape measure
- k. log book
- l. Go-flow bottle and DO collection SOP
- m. cooler and ice

- n. temperature monitor
- o. light and temperature controlled enclosure
- p. DO measurement plan

3.0 METHODS

3.1 Field method

1. Establish station location via GPS or visual reference.
2. Collect *in situ* water sample from a range of 0.5 to 1.0 meter off bottom at station using Go- flow bottle and following US EPA -ERLN dissolved oxygen collection SOP (See references)
3. Following US EPA -ERLN dissolved oxygen measurement SOP (See references) determine DO of *in situ* water.
4. Begin aerating *in situ* water as described in aeration SOP (See references).
5. Lower grab sampler and collect sediment sample following US EPA -ERLN Chemistry Sediment Grab SOP (See references) .
6. Push core tube into the grab sediment to produce a sediment sample in the core tube of three inches in depth.
7. Work a core cap through the surrounding sediment and on to the lower end of the core tube and cap. Place core in rack in cooler. Keep at 20°C.
8. Repeat grabs until 12 sediment core samples had been collected.
9. Measure aerated *in situ* water DO level to insure full DO saturation. Aerate further if required to reach full DO saturation.
10. Following DO SOP (See references) fill 12 DO bottles with aerated *in situ* water then fill core 12 sediment core samples with aerated *in situ* water, and seal all samples.
11. Measure aerated *in situ* water DO level and record. This record is DO time zero.
12. Maintain samples at 20°C in dark cooler and return to lab for analysis.

3.2 Lab Analysis

1. Maintain samples at 20°C and place in temperature and light controlled enclosure.
2. Prepare DO measurement equipment according to US EPA SOPs.
3. Follow DO measurement plan (See references) until end point is reached.
4. Enter DO data into PC And follow SOD Data Analysis Plan (See references) determine station specific SOD demand usage.

4.0 DATA ANALYSIS

The time sequence of DO measurements for the water and sediment samples are fit using linear regression to estimate the five day oxygen demand curve of water and sediment samples.

6.0 REFERENCES

1. US EPA-ERLN Dissolved Oxygen SOP
2. US EPA-ERLN Field DO Sampling SOP
3. US EPA-ERLN Sediment Collection SOP
4. DO Measurement Plan
5. SOD Data Analysis Plan

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1.0 OBJECTIVE

The purpose of this document is to describe the procedures used to measure dissolved oxygen with a Orion Dissolved Oxygen Meter Model 820 using probe model No. 082010.

2.0 NECESSARY MATERIALS AND EQUIPMENT

- a. Orion Dissolved Oxygen Meter Model 820.
- b. Orion Dissolved Oxygen Probe.
- c. Calibration sleeve.
- d. Natural seawater (NSW).
- e. Deionized water.
- f. Air pump.
- g. Delivery hose.

3.0 METHODS

3.1 DO Meter Set Up

1. Set up the Meter.
2. Plug the probe into the meter.
3. Turn on the meter (switch set to +).
4. If plug has been disconnected from the meter, allow probe to polarize for 20 minutes. If the probe has not been used recently, polarization may take up to one hour.
5. Aerate NSW (calibration water) for at least 15 minutes. This allows seawater to saturate via delivery hose attached to air pump.
6. Move Salinity Switch (rear panel) to the "ON" position.

7. Check the salinity of the saturated NSW.
8. Adjust the salinity value on the meter using the Scroll Key Pad.

3.2 Meter Calibration

1. Depress the mode Key Pad until "CAL" is displayed. The display will read "SAL", and then the last salinity value.
2. Adjust the salinity value to the appropriate calibration value using the Scroll Key Pad.
3. Briefly depress the Mode Key Pad, the display will read "CAL".
4. Depress quickly and release the Mode Key Pad. The display will show three dashes (---) followed by the slope of the electrode/membrane system. The slope should be between 0.7-1.2. Record slope in calibration note book. If the slope falls outside this range, or if an error message is displayed, refer to the SOP for DO meter maintenance: ETC DO-02.
2. Record the meter number, probe number and the slope in the calibration notebook.
3. Remove the probe from the calibration sleeve.
4. Depress the Mode Key Pad to select 'mg/L'.
5. Immerse the probe in the calibration NSW, making sure the stainless steel thermistor is covered, and stir gently.
6. When the display has stabilized, record the value in the calibration notebook. This value should be reasonably close to the value in Table 1 **Salinity vs. Temperature for D.O. Saturation**. If it is not, recalibrate and/or check the meter for any problems.
7. The meter is now ready to measure samples.

3.3 Measure DO in Test Chambers.

1. Immerse the probe in the sample, making sure the stainless steel thermistor is totally submerged.
2. Stir the probe gently.
3. When the display has stabilized record the value.
4. Between test chambers, rinse the probe with copious quantities of seawater water.
5. After the last measurement, turn off the meter, rinse the probe with DI water and return it to the calibration sleeve.

3.4 Storing DO Meter

1. For short term storage the probe does not need to be unplugged from the meter, it will remain polarized even when the meter is off.
2. The probe membrane must be kept moist, so be sure that the sponge inside the calibration sleeve has been saturated with DI water.
3. For long term storage, the membrane cap should be removed from the probe and stored as per Orion meter manual. The probe should be disconnected from the instrument, and the battery should be removed.

4.0 REFERENCES

1. Orion Dissolved Oxygen Meter Manual

Oxygen Solubility in Seawater
(values in mg/l)

Temp. (°C)	Salinity (ppt)								
	0	5	10	15	20	25	30	35	40
0	14.62	14.12	13.64	13.17	12.71	12.28	11.85	11.45	11.05
1	14.22	13.73	13.27	12.82	12.38	11.96	11.55	11.15	10.77
2	13.83	13.36	12.91	12.48	12.06	11.65	11.26	10.88	10.51
3	13.46	13.01	12.58	12.16	11.75	11.36	10.98	10.61	10.25
4	13.11	12.67	12.26	11.85	11.46	11.08	10.71	10.35	10.01
5	12.77	12.35	11.95	11.55	11.18	10.81	10.45	10.11	9.77
6	12.45	12.04	11.65	11.27	10.91	10.55	10.21	9.87	9.55
7	12.14	11.75	11.37	11.00	10.65	10.30	9.97	9.65	9.34
8	11.84	11.47	11.10	10.74	10.40	10.07	9.74	9.43	9.13
9	11.56	11.19	10.84	10.50	10.16	9.84	9.53	9.22	8.93
10	11.29	10.93	10.59	10.26	9.93	9.62	9.32	9.02	8.74
11	11.03	10.68	10.35	10.03	9.72	9.41	9.12	8.83	8.56
12	10.78	10.44	10.12	9.81	9.51	9.21	8.93	8.65	8.38
13	10.54	10.21	9.90	9.60	9.30	9.02	8.74	8.47	8.21
14	10.31	9.99	9.69	9.39	9.11	8.83	8.56	8.30	8.05
15	10.08	9.78	9.49	9.20	8.92	8.65	8.39	8.14	7.89
16	9.87	9.58	9.29	9.01	8.74	8.48	8.22	7.98	7.74
17	9.67	9.38	9.10	8.83	8.57	8.31	8.06	7.82	7.59
18	9.47	9.19	8.92	8.65	8.40	8.15	7.91	7.68	7.45
19	9.28	9.01	8.74	8.48	8.24	8.00	7.76	7.53	7.31
20	9.09	8.83	8.57	8.32	8.08	7.85	7.62	7.40	7.18
21	8.92	8.66	8.41	8.17	7.93	7.70	7.48	7.26	7.05
22	8.74	8.49	8.25	8.01	7.79	7.56	7.35	7.13	6.93
23	8.58	8.34	8.10	7.87	7.64	7.43	7.22	7.01	6.81
24	8.42	8.18	7.95	7.73	7.51	7.30	7.09	6.89	6.69
25	8.26	8.03	7.81	7.59	7.38	7.17	6.97	6.77	6.58
26	8.11	7.89	7.67	7.46	7.25	7.05	6.85	6.66	6.47
27	7.97	7.75	7.54	7.33	7.12	6.93	6.74	6.55	6.37
28	7.83	7.61	7.40	7.20	7.00	6.81	6.62	6.44	6.26
29	7.69	7.48	7.28	7.08	6.89	6.70	6.52	6.34	6.16
30	7.56	7.35	7.16	6.96	6.77	6.59	6.41	6.24	6.07
31	7.43	7.23	7.04	6.85	6.66	6.48	6.31	6.14	5.97
32	7.31	7.11	6.92	6.74	6.56	6.38	6.21	6.04	5.88
33	7.18	6.99	6.81	6.63	6.45	6.28	6.11	5.95	5.79
34	7.07	6.88	6.70	6.52	6.35	6.18	6.02	5.86	5.70
35	6.95	6.77	6.59	6.42	6.25	6.09	5.93	5.77	5.62
36	6.84	6.66	6.49	6.32	6.15	5.99	5.83	5.68	5.53
37	6.73	6.55	6.38	6.22	6.06	5.90	5.75	5.60	5.46
38	6.62	6.45	6.28	6.12	5.96	5.81	5.66	5.51	5.37
39	6.52	6.35	6.19	6.03	5.87	5.72	5.58	5.43	5.29
40	6.41	6.25	6.09	5.94	5.78	5.64	5.49	5.35	5.21

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1.0 OBJECTIVE

This document details the procedures for collecting and processing samples for suspended solids and chlorophyll a analyses.

2.0. NECESSARY MATERIALS AND EQUIPMENT

- a. General Oceanics Go-Flo or Niskin sampler
- b. 25 mm glass fiber filter (type GF-F, 0.7 μ m nominal pore size)
- c. 1 gallon Cubitainer
- d. sample bottle
- e. syringe filtering apparatus
- f. logbook.
- g. small zip-lock bag,
- h. forceps

3.0 METHODS

3.1 General Oceanics Go-Flo or Niskin Sampler Use

1. Attach the sampling bottle to the end of a calibrated, weighted line. Generally, this line will be the stainless steel winch cable.
2. Fully cock the sample bottle. This is a two-step process. First, cock the unit so that the ends are OPEN and the unit can be tripped by the messenger. Second, pull the rubber springs further until the ends of the bottle are CLOSED. The plastic balls on the tripping mechanism should be locked behind the release piston. When the bottle is set, the large balls on the ends of the sampler should be in the closed position. This prevents water from entering the bottle as it is immersed, thereby preventing contamination by the surface microlayer.

3. Lower the bottle into the water until the top is approximately 0.3 meters below the surface. Force the boat hook between the release piston and its protective cage, depressing the piston. This will trip the first mechanism, thereby opening the bottle. Lower the bottle to mid-depth then raise it and repeat until it has been rinsed by traveling at least 5 meters through the water column in the OPEN position. DO NOT ALLOW THE BOTTLE TO COME WITHIN APPROXIMATELY 0.3 METERS OF THE SURFACE IN THE OPEN POSITION. DOING SO COULD RESULT IN CONTAMINATION BY THE SURFACE MICROLAYER, WHICH COULD BE CONTAMINATED BY ENGINE EXHAUST.
4. Position the rinsed bottle approximately one meter below the surface, attach the messenger to the line and allow it to slide down the cable and trip the bottle.
5. Return the bottle to the boat, loosen the top valve, and drain approximately 200 ml of water through the bottom and into a 1 gallon Cubitainer to rinse it. Pour off this rinse and repeat for a total of three times, then drain the remainder into the Cubitainer.

3.2 Analyses of samples for suspended solids

1. Shake the Go-Flo bottle to assure no sediments have settled.
2. Fill the supplied 500 ml plastic container with water from the bottle, place an appropriate label on it, and place it on ice.
3. Record the sample number in the field logbook.

3.3 Chlorophyll a Determination

1. Install a 25 mm glass fiber filter (type GF-F, 0.7 μm nominal pore size) in the syringe filtering apparatus. Place several drops of a saturated MgCO_3 solution on the filter pad.
2. Fill the 60 ml syringe and apply pressure to force the water through the filter. Repeat as necessary until the filter pad takes on a greenish color. Following filtration, blow one syringe of air through the filter pad to dry it.
3. Record the volume filtered.

4. Using forceps, carefully remove the filter pad from the filtering apparatus, fold it in half twice (into quarters), and wrap it in aluminum foil. The filter pad must be handled with clean forceps as contact with the skin can cause the degradation of chlorophyll.
5. Place the foil-wrapped sample in a small zip-lock bag, write the sample number on the bag, and place label inside the bag. This is done because dry ice freezes the adhesive on the label and it may fall off. Record the sample number in the field note book.
6. Place the sample in a cooler on dry ice and keep frozen at -20°C.

4.0 REFERENCES

1. Standard Practices for Measurement of Chlorophyll Content of Algae in Surface Waters. ASTM Designation: D3731 -37 (Reapproved 1993)

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1.0 OBJECTIVE

This document details the procedures for operating the SEABIRD CTD Profiler during the collection water column salinity, conductivity, temperature, and dissolved oxygen.

The Sea-Bird CTD is a self-contained array of instruments capable of measuring a vertical profile of salinity, temperature, dissolved oxygen (DO) concentration, transmissivity (estimate of suspended solids concentration), fluorescence (estimate of chlorophyll a), and current velocity.

The core of the unit is a data logger which stores all data collected by the individual probes. The entire array can run off of internal batteries, therefore it does not require any electronic connection to the boat during operation. A deck unit is available if real time display of profile data is desired. Supplied with the instrument is the software required for communicating with the data logger and for uploading data to the on-board computer.

2.0 NECESSARY MATERIALS AND EQUIPMENT

1. Go-Flo bottle
2. Seabird CTD
3. log book
4. Seabird DO Probe
5. BOD bottle
6. 500ml plastic bottles
7. Seabird computer
8. Winkler kit
9. bucket

3.0 METHODS

3.1 Calibration

Each day, prior to sampling activities, a Quality Control (QC) check is performed. This can be performed at the dock, in a protected area, or on station depending on weather conditions and where it is most convenient for the crew. The procedure is as follows:

1. Connect the CTD to the end of the winch cable with a shackle, and **TIGHTEN THE PIN**. Use siezing wire to assure the shackle does not open. Make sure a "pinger" is attached to the unit.
2. Connect the CTD to the on-board computer and enter the Sea-Bird SeaSoft software. Erase all earlier data stored in the data logger, and prepare the CTD for data collection.
3. Manually turn the unit "on" and perform a QC check on the pH probe using a pH 7 reference buffer (make sure the grounding wire is in the pH buffer).
4. Lower the unit into the water to just below the surface. Real-time data should be displayed on the computer screen.
5. Allow the CTD to run for at least one minute for the DO probe to reach thermal equilibrium after being out of water.
6. Collect a water sample from the same depth as the intake for the CTD's pump. This sample is collected using a Go-Flo bottle deployed in the "open" position. This sample is used to verify the calibration of the CTD's DO probe. Freeze the computer screen at the time the water sample is collected.
7. Bring the unit back on board and turn "off".
8. Once retrieved, open the upper valve of the Go-Flo bottle and place the outlet hose into a 300 ml biological oxygen demand (BOD) bottle. The end of the hose should be approximately $\frac{3}{4}$ of the way down the bottle. Allow the water to enter the bottle **SLOWLY** and overflow (at least two volumes i.e., 600 ml). Repeat the procedure for an additional bottle. Also fill two 500 ml plastic bottles. The samples are then analyzed for DO using the Winkler technique (Appendix B), and for suspended solids determinations (Appendix C). Titrations should be conducted as soon as possible. Check the salinity of the sample (to the nearest 1 ppt) using a refractometer and the temperature using a stem thermometer.

9. Compare CTD values frozen on the computer screen in step 6 with those measured in steps 2 and 8 for pH, salinity, and temperature. If they do not agree within 0.5 pH unit, 2 ppt, and 2 °C, respectively, the process must be repeated. If, after a second attempt, the unit still produces erroneous numbers, calibration will be necessary. This procedure is described in Appendix H. If the value obtained from the Winkler does not agree with the CTD value to within 1 mg/l, all DO data collected that day are flagged.
10. The investigator always has the option of proceeding, even if the QC values exceed the acceptable error; however, he/she should record the event in the field logbook.
11. Reset the instrument so it is ready for the first station, disconnect the data cable, and install the dummy plug and locking sleeve.

3.2 Data Collection

At each station, the general procedures for collection of data are as follows.

1. Connect the CTD to the end of the winch cable with a shackle, and **TIGHTEN THE PIN**. Use siezing wire to assure the shackle does not open. Make sure a "pinger" is attached to the unit.
2. Swing the instrument over the side, turn it on, and lower it to just below the water surface.
3. After allowing the instrument to reach thermal equilibrium (at least one minute), collect a surface water sample with a Go-Flo bottle or a bucket, and lower the CTD through the water column at a rate of approximately one meter per second until it reaches the bottom. When used in real time, the depth readout can be used to "observe" the unit as it descends. Prevent the CTD from impacting the bottom by stopping its descent when it is approximately one meter or less from the bottom. Raise the unit to the surface, turn it off, and bring it back into the boat. Process the water sample collected for chlorophyll a and suspended solids as described in the Appendices.
4. Following completion of the CTD cast, connect the CTD to the on-board computer and upload the data using the Sea-Bird software. This software bin averages the data over one-meter intervals, and should be instructed to use only the downcast data. Upcast data are generally not as accurate as the downcast data because of the orientation of the

probes.

5. After data are stored in the on-board computer, view the data on the screen using the Sea-Bird software. If, for any reason, the cast was not successful, all steps are repeated up to a total of three attempts.
6. Record appropriate data on the DO data sheet. This includes the bottom salinity and if the bottom DO is zero.

3.2 Safety Considerations

The CTD is fairly heavy; therefore, care should be taken when deploying or retrieving this unit from the end of the boom under adverse weather conditions. The only other danger to the user is from the operation of the winch. Of course, care should be taken not to damage the instrument.

3.3 Quality Assurance

As the CTD is a delicate electronic instrument, certain precautions are necessary to assure proper operation. All instructions should be followed closely. QC calibration checks must be performed daily. If the instrument falls out of calibration, it must be re-calibrated before any data are collected.

The transformation of transmissivity and fluorescence voltage readings to suspended solids and chlorophyll is dependent on the characteristics of the particulates in the water column. The chlorophyll and suspended solids samples will be used in determining these relationships.

3.4 Contingencies

1. If the water depth is too shallow (≤ 3 meters) to obtain a profile, suspend the unit just above the bottom and collect data for two minutes (following a one minute warm-up period). This must be noted in the field notebook.
2. If the CTD fails to function properly, the hand-operated profiling instrument should be used to obtain a profile. Record data at one meter intervals in the field notebook.
3. Any time a contingency plan is initiated, the Field Coordinator (FC) must be notified. In the case of equipment failure, the FC should be notified immediately so arrangements can be made for shipping back-up equipment.

3.5 Sampling Design

Physical measurements using a SEA-BIRD CTD/Marsh McBirney Current Profiler will be taken at stations selected on the basis of results obtained from other surveys. When logistical considerations dictate that the instrument be deployed in tandem with other instruments/activities, the planned configuration will be to place the CTD above and upstream of other instruments to allow undisturbed flow measurements.

3.6 Sampling Parameters and Frequency

All sampling parameters are measured at a rate of 24 sec^{-1} . Given approx. 30 sec of bottom time, about 700 measurements will be made. All calculations are done on data stored in binary format. Sampling parameters used to determine current speed include measures of speed in X and Y components and instrument tilt in X and Y components. After tilt correction, current speed is determined by sums-of-squares method. Current direction is calculated from tilt corrected X and Y components after rotation to earth coordinates (North and East, respectively). Rotation is based on internal flux-gate compass measurements. After rotation, current direction is determined by sums-of-squares method.

E. Parameter Table

PARAMETER	RANGE	ACCURACY	RESOLUTION	RESPONSE TIME
Temperature	-5 to +35°C	0.004°C/yr	0.0003°C	0.07 sec
Conductivity	0 to 7 S/m	0.001 S/m/mo	0.00004 S/m	0.07 sec
Depth	0 to 100 psi	0.1 psi	0.05 psi	0.01 sec

4.0 DATA QUALITY REQUIREMENTS AND ASSESSMENTS

- 4.1 Accuracy. Accuracy of measured parameters is based on calibrations done on probes by the Northwest Regional Calibration Center. Sensors are calibrated by subjecting separate modules to known physical conditions, and measuring the sensor responses. Sensors are supplied fully calibrated with coefficients printed on calibration certificates. Sensors may be returned to NRCC for re-calibration. If possible, measurements will be made in conjunction with the deployment of Battelle CTD-current meter moorings for inter-calibration.
- 4.2 Precision. Precision will be measured as the standard deviation of all data collection. Given 30 seconds bottom time, as many as 500-700 measurements can be taken. If, however, a long interval between retrievals is required (e.g. > 5-7 min.), scan rate will be reduced accordingly. At 1 sample/sec., time between retrievals can be > 2 hours.
- 4.3 Representativeness. Using results of current mooring deployments, an independent assessment of the above bottom energy field will be obtained to which our current velocities can be compared. The degree of agreement between methods will determine the extent to which our current data is representative of actual field conditions.
- 4.4 Comparability. The collection of current measurements in the inshore regions of Cameron LA are previously unavailable. Thus, no previous studies exist to which this approach can be compared. However, many studies exist in which currents in offshore regions have been measured. Thus, a sound basis for data interpretation is available in the literature.
- 4.5 Completeness. Based on results of side scan surveys, several regions within in each site will be chosen based on bedform morphologies in order to reduce the total area to be sampled. Within each region of similar bedform type, several stations will be selected. By this approach, we expect that each site will be fully characterized in regard to potential places for dredged material disposal.

5.0 DOCUMENTATION, DATA REDUCTION AND REPORTING

5.1 Documentation. Raw data retrieved from the CTD instrument is saved on floppy disks in binary format. Data from each deployment is stored in a separate data file along with initial and final date, time, latitude, longitude and depth. Backups of floppy disks will be made aboard ship and stored separately. A logbook containing station no., date, time, latitude, longitude, depth and systems operator will also be kept.

5.2 Data Reduction and Reporting. Raw data from each instrument deployment will be converted into engineering units and split into separate files for each station. Time and depth records will be used to delineate stations. Each file will be tagged with station no., date, time, latitude, longitude, depth and systems package (LOTUS 1-2-3) for further calculations.

6.0 QA Procedures for Calibration of SEABIRD probes

6.1 Dissolved oxygen

Dissolved oxygen will be measured using polarographic probes such as that attached to the SeaBird CTD instrument. The probe is rated by the manufacturers as being accurate to 0.2 ppm. The probe will be calibrated prior to deployment using the saturated air calibration procedure recommended by the manufacturers. In addition, a supersaturated solution of sodium sulfite will be used to provide a zero calibration check for either probe. All calibration values will be recorded prior to deployment of the probe.

The calibration of the probe attached to the CTD will be checked once each day by taking a simultaneous water sample and measuring dissolved oxygen concentration by Winkler titration. If the Winkler results and those obtained from the probe differ by greater than 0.5 ppm, the probe must be checked for malfunctions, re-calibrated, then re-checked for calibration before it can be redeployed. All previous data (i.e., since the last successful calibration check) will be flagged and will be reviewed for validity prior to data release.

6.2 Salinity

Salinity will be measured using the SeaBird CTD profiling recording probe which is rated by the manufacturer as being accurate to 1 percent. Salinity

meters are calibrated by the manufacturer; this calibration will be checked once each day using a refractometer. It is expected that the probe on the CTD will be more accurate than the refractometer; therefore, the refractometer measurement will act only as a gross check on the operation of the probe. However, if the refractometer reading differs from the probe value by greater than 1 part per thousand, the CTD instrument will be checked thoroughly and a determination made of the need for recalibration.

6.3 Temperature

Temperature will be measured using the SeaBird CTD profiling recording probe which is rated by the manufacturer as being accurate to 0.2 °C. The temperature sensor on the probe will be calibrated by the manufacturer using a National Bureau of Standards [NBS] certified thermometer, and the calibration value recorded prior to probe use. Probes will be tested for calibration stability each day using a thermometer. Drift from the original calibration will be used as a criteria for data quality acceptance and as a data flagging criteria. If calibration results differ from the original calibration by greater than 0.5 °C, the data will be flagged as being outside the quality control criteria and will then be reviewed for validity prior to data release.

6.4 pH Measurements (if available)

Measurements of pH will be taken with the SeaBird CTD. The instrument will be calibrated to pH 7 and pH 10 as described in Strobel et al. (in preparation). Following calibration, a QC check will be performed using an intermediate range buffer solution (pH 8 is suggested). The QC check should be within 0.2 limits, the instrument calibration should be checked. Quality control checks should be performed and recorded prior to and following deployment of the CTD.

6.5 Fluorometry

In situ fluorescence will be measured using a Sea Tech fluorometer attached to the Seabird CTD. The optical filters used in this fluorometer have been selected for optimum measurement of chlorophyll a fluorescence. Prior to each deployment, the instrument will be checked to insure that it is functioning properly, following the manufacturer's instructions. At each station, two duplicate surface water samples will be collected simultaneously with deployment of the instrument. A pre-determined volume of the water sample

will be filtered on-board and the filter frozen for subsequent determination of chlorophyll a concentration. Over time, this will provide a means of calibrating each fluorometer (i.e., converting its fluorescence readings into chlorophyll a concentrations). At every tenth station, an additional set of water samples, identical to the first, will be filtered for additional chlorophyll a measurements. These duplicate measurements should not differ by more than 10%. Failure to achieve this precision goal will result in a thorough review of the field and laboratory procedures, to determine the cause of the discrepancy and eliminate it.

6.5 Transmissometry

A Sea Tech 5 or 10 cm pathlength transmissometer will be used to provide in situ measurements of beam transmission and the concentration of suspended matter at each station occupied. The manufacturer's procedures for internal calibration in air and instrument check-out must be completed prior to each study.

In general, optical devices such as transmissometers are useful for determining suspended particle concentrations in near coastal waters as long as the nature of the suspended matter does not change much from region to region. Each transmissometer will be calibrated based on field measurements of suspended particle concentrations. Suspended particle concentrations will be determined in surface water samples taken simultaneously with the transmissometer reading. A known volume of the surface water sample will be filtering on board and frozen for later laboratory measurements of suspended solids (i.e., particle) concentration. At every tenth station, a second volume of the water sample, identical to the first, will be filtered to provide duplicate suspended solids measurements. These duplicate measurements should not differ by more than 10%. Failure to achieve this precision goal will result in a thorough review of the field and laboratory procedures, to determine the cause of the discrepancy and eliminate it.

7.0 REFERENCES

1. SBE25 Sealogger CTD Operating Manual. April 1990. Seabird Electronics, Inc. 1808 136th Place NE Bellevue, Washington 98005. (206)-643-9866

TOTAL AMMONIA ANALYSIS USING THE SALICYLATE-HYPOCHLORITE METHOD

1.0 OBJECTIVE

- 1.1 This document describes the methods used to analyze total ammonia in overlying and porewater samples.
- 1.2 The salicylate-hypochlorite method is used to measure the level of total ammonia found in seawater in overlying and porewater samples.

2.0 SAFETY

- 2.1 Environmental samples may contain hazardous biological or chemical constituents. Latex gloves (rinsed before wearing), poly laminated labcoat, and safety glasses are to be worn.

3.0 MATERIALS

- 3.1 Ammonia standard (1000 \pm 5 ppm as N)
- 3.2 Sodium salicylate
- 3.3 Sodium nitroprusside
- 3.4 Sodium hydroxide
- 3.5 Sodium citrate
- 3.6 Clorox[®] bleach
- 3.7 1L volumetric flask
- 3.8 100ml glass graduated cylinder
- 3.9 Five(5) 125ml glass Erlenmeyer flasks
- 3.10 20ml test tubes
- 3.11 Brown bottle 1L
- 3.12 Eppendorf repeater pipette
- 3.13 1.25ml Eppendorf pipette tip (1=25 μ l)

3.14 12.5 ml Eppendorf pipette tip (1 = 250 μ l)

3.15 50ml Eppendorf pipette tip (1 = 1ml)

3.16 10ml Oxford pipettor

3.17 1ml variable Oxford pipetter

3.18 Spectrophotometer

3.19 50 ml Syringe

3.20 Magnetic stir plate

3.21 Magnetic stir bars

3.22 Automatic scale

4.0 METHODS

4.1 **Prepare Reagents.**

(Note: Salicylate-catalyst solution and Alkaline-citrate solution can be made beforehand and stored for when needed. Alkaline-hypochlorite solution should be made up immediately before use.)

4.1.1 Prepare Salicylate-catalyst solution.

1. Weigh 440g sodium salicylate.
2. Weigh .28g sodium nitroprusside.
3. Place in 1L volumetric flask.
4. Dissolve and stir in 1L deionized water.
5. Transfer to brown 1L bottle.
6. Store at 5°C. (Stable solution for 3 months. Blank and sample absorbances will increase with solution age.)

4.1.2 Prepare Alkaline-citrate solution.

1. Weigh 18.5g sodium hydroxide.
2. Weigh 100g sodium citrate.
3. Place in 1L volumetric flask.
4. Dissolve and stir in 1L deionized water.
5. Store at 5°C. (Stable indefinitely)

4.1.3 Prepare Alkaline-hypochlorite Solution.

1. Dilute 1ml Clorox® in 9ml Alkaine-citrate per 10ml solution needed.
(Note: This must be used within 1 hour of preparation.)

4.2 Prepare Ammonia Standard Curve.

1. Label 125ml Erlenmeyer flasks 0.0, .25, .50, 1.0, and 2.0 respectively.
2. Measure 100ml deionized water into each flask.
3. Add 25 μ l ammonia standard to the .25 flask and swirl. (Set 1.25ml Eppendorf tip to '1' and deliver to flask.)
4. Add 50 μ l ammonia standard to the .50 flask and swirl. (Set 1.25ml Eppendorf tip to '2' and deliver to flask)
5. Add 100 μ l ammonia standard to the 1.0 flask and swirl. (Set 1.25ml Eppendorf tip to '4' and deliver to flask)
6. Add 200 μ l ammonia standard to the 2.0 flask and swirl. (Set 1.25ml Eppendorf tip to '4' and deliver twice(2x).)
7. Transfer 10ml of solution from the 0.0 flask into each of three test tubes.
8. Repeat for each concentration so there are three replicates for each concentration.

4.3 Prepare samples for ammonia analysis.

1. Label test tubes to correspond with sample chambers. (One replicate for each sample.)
2. Add 9ml deionized water to each test tube using 10ml Oxford pipette if sampling for overlying ammonia.
Add 9.5ml deionized water to each test tube using 10ml Oxford pipette if sampling for porewater ammonia.
3. Transfer 1.0ml overlying water from the first test chamber to corresponding test tube.
4. Remove 1.0 ml overlying water from the next chamber in series and discard.
5. Remove 1.0ml overlying water from test chamber and transfer to corresponding test tube.
6. Repeat steps 4 and 5 for each test chamber in series.
7. Gently swirl test tubes to mix.

4.4 Turn on spectrophotometer at least 20 minutes prior to use.

4.5 Set up spectrophotometer.

1. If allowing gravity to draw the sample through the pour thru cell, make sure that the tubing exiting the cell is attached to the base assembly and the drain hose runs to a waste vessel.
2. If manually drawing the sample through the pour thru cell, remove the tube exiting the cell from the base assembly and attach to the 50ml syringe.

4.6 Flush the spectrophotometer.

1. Pour 25ml deionized water into funnel on the spectrophotometer.
2. Draw water into the pour thru cell. (If not using the syringe, gravity will draw the water through the cell.)

4.7 Press the zero and absorbance (abs) buttons to attain a stable zero reading.

- 4.8 Set spectrophotometer to 640nm.**
- 4.9 Add 1.25ml Salicylate-catalyst solution to each test tube(Standard curve and samples).**
(Set 12.5ml Eppendorf pipette tip for '5' and deliver to test tube.)
- 4.10 Add 2.0ml Citrate-hypochlorite solution to each test tube.**
(Set 50ml Eppendorf pipette tip for '2' and deliver to test tube.)
- 4.11 Cover test tubes with black plastic.**
- 4.12 Uncover test tubes after at least 30 minutes but no longer than 3 hours.**
- 4.13 Set up standard curve**
 - 4.13.1 Pour first blank (0.0) replicate through funnel.
 - 4.13.2 Draw through pour thru cell
 - 4.13.3 Read absorbance value and record on data sheet.
 - 4.13.4 Repeat steps 4.13 through 4.15 for each replicate.
 - 4.13.5 Repeat steps 4.13 through 4.16 for each concentration in the standard curve from lowest to highest.
 - 4.13.6 Flush system with deionized water after last 2.0 replicate until absorbance reads zero.
- 4.14 Read sample absorbances**
 - 4.14.1 Pour sample test tube through funnel.
 - 4.14.2 Draw through pour thru cell.
 - 4.14.3 Read absorbance value and record on data sheet.
 - 4.14.4 Repeat steps 4.14.1 through 4.14.3 for each sample in series.
- 4.15 Flush system with deionized water (50-100ml) until spectrophotometer reads zero.**
- 4.16 Wipe down spectrophotometer with wet cloth and clean work area.**
- 4.17 Turn off spectrophotometer.**

**48 HOUR EMBRYO/LARVAL DEVELOPMENT TEST USING THE SEA URCHIN
*ARBACIA PUNCTULATA***

1.0 OBJECTIVE

- 1.1 This document describes the methods used to measure the toxicity of effluent and receiving waters to the larvae of the sea urchin *Arbacia punctulata*.
- 1.2 The purpose of the larval development toxicity test is to determine the concentration of a test treatment that inhibits survival and development relative to that of the control.

2.0 SAFETY

- 2.1 Environmental samples may contain hazardous biological or chemical constituents. Latex gloves (rinsed before wearing), tyvek labcoat, and safety glasses are to be worn.
- 2.2 Embryos preserved in formalin must be examined under a fume hood.

3.0 MATERIALS

- 3.1 Air pump
- 3.2 Plastic 1 ml pipettes
- 3.3 Centrifuge, bench top, variable speed
- 3.4 Fume hood.
- 3.5 Dissecting microscope with detachable light
- 3.6 Compound microscope
- 3.7 Sedgwick-Rafter counting chamber
- 3.8 Hemacytometer
- 3.9 Count register, 2-place
- 3.10 Ice bucket
- 3.11 Capped centrifuge tubes, conical, 50 ml, plastic



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- 3.12 18 oz, tall, glass jar
 - 3.13 Two large crystallization dishes
 - 3.14 Wash bottles filled with deionized water and natural sea water
 - 3.15 Transformer, 10-12 volt, with steel electrodes
 - 3.16 Two syringes: 1cc (1ml), and 10cc (10 ml), with 18 gauge, blunt-tipped needles (tips cut off).
Or an acceptable substitute (i.e. a modified pipette tip attached to the syringe with 1/8 inch silastic tubing)
 - 3.17 5 ml, automatic pipette
 - 3.18 1 ml, adjustable pipette
 - 3.19 Permanent marker
 - 3.20 Sea urchins, 4 or 5 of each sex
 - 3.21 Scintillation vials, 20 ml, disposable
 - 3.22 250 ml glass exposure chamber
 - 3.23 Plastic Plunger
 - 3.24 20 ml grid-type petri dish
 - 3.25 Formalin, 5% buffered in sea water, filtered
 - 3.26 Acetic acid, reagent grade, 10% in sea water
 - 3.27 Hypersaline brine (as needed)
 - 3.28 Gloves, lab coat, and safety glasses
 - 3.29 Data sheets (attached)
 - 4.0 METHODS
 - 4.1 Prepare samples.



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- 4.1.1 Adjust salinity of sample to 28 to 30 ppt with hypersaline brine if necessary (see ETC SOP). Prepare dilutions if necessary.
 - 4.2 **Fill test chambers.**
 - 4.2.1 Dispense 200 mls of sample or dilution of sample into each of three replicate exposure chambers.
 - 4.3 **Prepare gamete dilution vials.**
 - 4.3.1 Label and fill the sperm dilution vials as follows:
 - A: 19 mls of NSW
 - B: 10 mls of NSW
 - C: 10 mls of NSW
 - D: 10 mls of NSW
 - E: 4 mls of NSW
 - 4.3.2 Place vials A, B, and D on ice for later use.
 - 4.3.3 Label and fill four egg dilution vials with 9 mls of NSW and set aside.
 - 4.4 **Collect the eggs.**
 - 4.4.1 Select four female urchins and place in large crystallization dish, barely covering the tests with sea water.
 - 4.4.2 Direct microscope light on urchins to better view gamete release.
 - 4.4.3 Stimulate the release of eggs by touching the test with electrodes from the transformer.
NOTE: Do not let the electrodes touch the genital pore or gametes.
 - 4.4.4 Collect eggs from at least three of the females in the dish using a 10 cc syringe with a blunted tip.
 - 4.4.5 Remove the needle from the syringe before adding the eggs to a 50 ml conical centrifuge tube containing several mls of control seawater.
 - 4.4.6 Bring contents of centrifuge tube to maximum volume by adding control seawater.
 - 4.4.7 The egg stock may be held at room temperature for several hours before use and may be prepared during sperm exposure to sample or dilution of sample.
 - 4.5 **Collect the sperm.**



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- 4.5.1 Select four males and place in large dish, barely covering the urchins with sea water.
- 4.5.2 Direct microscope light on the urchins to better view the release of gametes.
- 4.5.3 Stimulate the release of sperm by touching the test with electrodes from the transformer.
NOTE: Do not let the electrodes touch the genital pore or gametes.
- 4.5.4 Collect sperm from at least three of the males, using a 1 ml disposable syringe fitted with an 18-gauge, blunt tipped needle. Collect until syringe is full.
- 4.5.5 Keep the syringe containing pooled sperm sample on ice.
- 4.5.6 The sperm should be used within 1 hour of collection.
- 4.6 Prepare the sperm.**
- 4.6.1 Estimate the sperm concentration by preparing dilutions of 1:50, 1:100, 1:200, and 1:400, using 30 ppt seawater. NOTE: All sperm vials should be maintained on ice before starting the test.
1. Add 1 ml of collected sperm to 19 ml of seawater in Vial A. Cap Vial A and mix by inversion.
 2. Add 10 mls of sperm suspension from Vial A to 10 mls of seawater in Vial B. Cap Vial B and mix by inversion.
 3. Add 10 mls of sperm suspension from Vial B to 10 mls of seawater in Vial C. Cap Vial C and mix by inversion.
 4. Add 10 mls of sperm suspension from Vial B to 10 mls of seawater in Vial D. Cap Vial D and mix by inversion.
 5. Discard 10 mls from Vial D. (The final volume of all sperm suspensions is 10 mls).
- 4.6.2 Make a 1:2000 killed sperm suspension and determine the sperm/ml (SPM)
1. Add 10 mls 10% acetic acid in seawater to Vial C. Cap Vial C and mix by inversion.
 2. Add 1ml of killed sperm from Vial C to 4 mls seawater in Vial E. Mix by gentle inversion.
 3. Add sperm from Vial E to both sides of the hemacytometer. Let the sperm settle for 15 minutes.
 4. Count the number of sperm in the central 400 squares on both sides of the hemacytometer using a compound microscope (400X).
 5. Average the counts from the two sides and calculate the SPM using the calculation: SPM in Vial E = 10^4 x average count from Vial E.

4.6.3 Calculate the SPM in all other suspensions using the SPM in Vial E.

1. SPM in Vial A = 40 x SPM in Vial E.
2. SPM in Vial B = 20 x SPM in Vial E.
3. SPM in Vial D = 5 x SPM in Vial E.
4. SPM in original sperm sample = 2000 x SPM in Vial E.

4.6.4 Select the vial with a sperm concentration greater than and closest to 5×10^7

4.6.5 Using the following calculation, dilute the sperm concentration of the chosen vial to 5×10^7 .

1. Actual SPM/ (5×10^7) = dilution factor (DF).
2. $((DF) \times 10) - 10$ = mls of seawater to add to vial.

4.7 Prepare the eggs.

4.7.1 Using a tabletop centrifuge, wash the pooled eggs twice with control seawater.

NOTE: This can be done while waiting for the sperm to settle on the hemacytometer.

1. Spin for two minutes at lowest possible setting.
2. Carefully pour off the overlying water.
3. Add more control seawater and spin again.

4.7.2 If the wash water becomes red, the eggs have lysed and must be discarded.

4.7.3 Remove the final wash water and refill the tube with control water.

4.7.4 Transfer the washed eggs from the centrifuge tube to a beaker containing a small volume (about 50 mls) of control water by gently inverting the tube to suspend the eggs and carefully pouring the contents into the beaker.

4.7.5 Estimate the egg concentration by preparing a 1:10 dilution using control seawater. NOTE: The desired egg stock concentration is 3500 ± 350 eggs/ml, the desired count for the dilutions is 350 ± 35 eggs/ml.

1. Dilute the egg stock by adding enough control water to the beaker to bring the egg stock to a volume of 200 ml.
2. Suspend the egg stock using gentle aeration.
3. Cut the point from a 1 ml pipette tip and use it to transfer 1 ml of suspended egg stock into two vials containing 9 mls of control water
4. Mix the contents of each vial by inversion and transfer 1 ml of eggs from each vial to a Sedgwick-Rafter counting chamber.

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5. Count all of the eggs in the chamber using a dissecting microscope.
 6. Calculate the 'egg count' by averaging the counts from both vials
- 4.7.6 Calculate the egg stock concentration using the equation: $\text{Eggs/ml} = 10 \times (\text{egg count})$.
 - 4.7.7 Dilute the egg stock to 3500 ± 350 eggs/ml.
 1. If the egg count is equal to or greater than 350: $(\text{egg count}) - 350 = \text{volume (ml) of control water to add to egg stock}$.
 2. If the egg count is less than 350, allow the eggs to settle and remove enough control water to concentrate the eggs to greater than 350, repeat the count, and dilute the egg stock as above. NOTE: It requires 18 ml of an egg stock solution for each test with a control and five exposure concentrations (three replicates).
 - 4.7.8 After diluting or concentrating the egg stock confirm the final egg count by repeating step 4.7.5.
 1. Suspend the egg stock using gentle aeration.
 2. Cut the point from a 1 ml pipette tip and use it to transfer 1 ml of suspended egg stock into two vials containing 9 mls of control water.
 3. Mix the contents of each vial by inversion and transfer 1 ml of eggs from each vial to a Sedgwick-Rafter counting chamber.
 4. Count all of the eggs in the chamber using a dissecting microscope.
 5. Calculate the 'egg count' by averaging the counts from both vials.
- 4.8 Fertilize the eggs.**
- 4.8.1 Mix the egg stock well and subsample 100mls.
 - 4.8.2 Pour the subsample into a clean beaker labeled 'embryo suspension'.
 - 4.8.3 Within 1 hour of collection, add 1.75 mls of the proper sperm dilution to the beaker and mix well. NOTE: This will result in an egg:sperm ratio of 1:2500, which should allow acceptable egg fertilization.
 - 4.8.4 Allow 1 hour for fertilization.
- 4.9 Start the test.**
- 4.9.1 Mix the embryo suspension (3500 eggs/ml), using gentle aeration.



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- 4.9.2 Add 1 ml of egg suspension to each 100 mls of test solution in each exposure chamber using a cut, 1 ml pipette tip.
- 4.9.3 Determine initial counts (for survival endpoint) by gently suspending the test media in each control chamber using a plunger.
1. Sub-sample two 10 ml aliquots from each of the control chambers into two 20 ml scintillation vials.
 2. Preserve the samples by adding 2 ml of 2.5% buffered formalin and in seawater to each vial.
 3. Count all of the fertilized eggs in each vial. Record and average the counts to determine the actual number of embryos added at test initiation.
- 4.9.4 Incubate test chambers for 48 hours at $20 \pm 1^\circ\text{C}$.
- 4.9.5 Record physical data daily .
- 4.10 Terminate the test.**
- 4.10.1 Gently suspend the test media in each exposure jar using a plunger.
- 4.10.2 Sub-sample two 10 ml aliquots from each chamber into two 20 ml scintillation vials.
- 4.10.3 Preserve the samples by adding 2 ml of 2.5% buffered formalin and Rose Bengal in seawater to each vial.
- 4.11.4 Cap each vial tightly.
- 4.11 Evaluate the test.**
- NOTE: Vials may be evaluated immediately or they can be stored refrigerated for as long as one week.
- 4.11.1 Gently mix each vial by inversion.
- 4.11.2 Carefully pour the entire content into a 20 ml grid-type petri dish.
- 4.11.3 Observe the embryos using a compound microscope (40-100X) under a fume hood.
- 4.11.4 Count the total number of live larvae in each vial. Distinguish between normal and abnormal larvae. NOTE: Do not include the number of dead animals in either total.

- 4.11.5 Record the number 'normal' and 'abnormal' to determine development relative to the control. The total number of larvae is used to determine percent survival relative to the control and test initiation.



Standard Operating Procedure

SOP MI-011
Microinorganics, Inc.
Marine Tissue/Metals

Wet Digestion Procedure for the Preparation of Marine Tissue Samples for Trace Metal Analysis.

Contact:

Mr. J.D. Cullen
Microinorganics, Inc.
16 Reactor Road
Narragansett, RI 02882

Materials:

1. Quartz beakers
2. Teflon Watch Glasses
3. Hot Plate
4. Homogenizer, with plastic mixing bowls, and custom made titanium cutting blade.
5. Instra-Analyzed Nitric Acid (J.T. Baker)
6. Instra-Analyzed Hydrochloric Acid (J.T. Baker)
7. Ultrex Hydrogen Peroxide (J.T. Baker)
8. Nuclepore Membranes (0.4 μ m pore size, 25mm diameter)
9. Swinnex Filter Holders (Millipore Corp.)
10. Polypropylene syringes
11. Plastic spatulas
12. Top-loading balance
13. Drying Oven
14. Polypropylene bottles (acid-cleaned) and vials

Procedure:

1. Frozen whole tissues are removed from the shell using a plastic spatula, and placed in a tared, acid-cleaned mixing bowl. The total weight of the tissue sample is determined after shucking all of the tissue samples from a batch into the mixing bowl.
2. The mixing bowl is placed on the homogenizer, the titanium cutting blade inserted, and the sample is homogenized for 15 to 30 minutes depending on the size of the sample.

APPENDIX B

QUALITY ASSURANCE AND QUALITY CONTROL PROTOCOLS

APPENDIX B

**Quality Assurance Project Plan
for the
Offshore Ecological Risk Assessment
at the
Naval Education and Training Center
Newport, Rhode Island**

- B.1 Chemistry**
- B.2 Biology and Toxicity**

**Graduate School of Oceanography
University of Rhode Island
Narragansett, RI 02882-1197**

and

**Science Applications International Corporation
Narragansett, RI 02882**

March 1995

B.1 CHEMISTRY

INTRODUCTION

This Quality Assurance Project Plan (QAPjP) provides Quality Control / Quality Assurance guidelines to be followed during the chemical analysis of sediment, tissue and pore water samples collected as part of the Ecological Risk Assessment at NETC. This plan was developed by following the QA Project Plan prepared by the Environmental Research Laboratory, Narragansett (U.S. EPA) for an Estuarine Ecological Risk Assessment at the Portsmouth Naval Shipyard, Kittery, Maine (EPA, 1993).

Project Description

The project includes a sampling program to assess the bioaccumulation potential of inorganic and organic contaminants in native and deployed organisms from selected sites at NETC. In addition, sediment (surface grabs and cores) and pore water samples will be collected and analyzed in order to evaluate the geochemical assimilation and bioavailability of the contaminants. Potential source samples will also be examined for the possible presence of contaminants that may be used as chemical markers.

Objectives

The objective of this project is to perform chemical analysis of sediments, tissues and pore water samples collected as part of the Offshore Ecological Risk Assessment at NETC.

PROJECT ORGANIZATION AND RESPONSIBILITIES

The work assignment organization chart (Figure 1) shows key personnel associated with the chemical work assignment. Responsibilities of these personnel are listed below.

Professor James Quinn (GSO) will function as the overall Program Manager. He will be directly responsible to HNUS for the overall conduct of the program and for the quality and timely submission of all deliverables.

Dr. Robert Cairns (GSO) will be the Work Assignment Manager for organics. He will oversee and coordinate all of the activities of the organic chemistry laboratory personnel. He will assist Dr. Quinn in the data synthesis, interpretation, QA/QC and in the preparation and submission of reports and plans to HNUS.

Professor John King (GSO) will be the Work Assignment Manager for inorganics. He will oversee and coordinate all of the activities of the inorganic laboratory personnel. He will be assisted by Mr. Paul Gangemi (GSO) in the data synthesis, interpretation, QA/QC and in the preparation and submission of reports and plans to Professor Quinn.

Ms. Andrea Helmstetter (SAIC) will be the Quality Assurance Officer. She will work with Professors Quinn and King to ensure that the data generated under this work assignment are in compliance with HNUS quality assurance plans and programs. She will also be responsible for the auditing of the laboratories for compliance with data and analytical protocols as specified in the QAPJP, and for the maintenance of all QA materials.

QUALITY ASSURANCE/QUALITY CONTROL (QA/QC) REQUIREMENTS

The characteristics that define the quality of data are accuracy, precision, completeness, comparability and representativeness and method detection limit. These characteristics are defined below. Key elements for quality control of chemical analysis are given in Table 1.

ACCURACY

Accuracy is the closeness of agreement between an observed and accepted value. Accuracy is verified by the analysis of reference materials, intercalibration samples, internal standards, procedural blanks, initial calibration, calibration checks and matrix spikes.

Reference Materials

Reference Materials (as Standard Reference Materials (SRMs) or Certified Reference Materials (CRMs)) are analyzed to determine the efficiency and accuracy of the method. These Reference Materials have been certified for concentrations of the analytes of interest by a recognized authority. Analysis of an SRM will accompany every 20 field samples of sediment or tissue for organic or metals analysis (e.g. NIST 1941a Sediment or 1974a Tissue for organic contaminants in sediment and tissue, respectively). Percent recovery for analytes of interest is calculated by the following:

$$\% R = \frac{C_1}{C_2} \times 100$$

% R = percent recovery
 C1 = measured value
 C2 = certified value

Intercalibration Exercises

The GSO participates in the yearly intercalibration exercises sponsored by the NOAA National Status and Trends Program for the measurement of polychlorinated biphenyls (PCBs), organochlorine pesticide (OCPs), polycyclic aromatic hydrocarbons (PAHs), and selected metals. These are meant to assess the laboratory's performance on a continuous basis. Each exercise involves the blind analysis of different representative matrices; two or three different NS&T exercises are conducted over the course of a year by the National Institute of Standards and Technology.

Internal Standards

Internal surrogate standards are reference compounds selected to be representative of the various classes of analytes. They are added immediately prior to the extraction of each sample. Their recovery, as measured by external recovery standards added just prior to instrumental analysis, will also be used as a measure of method performance.

Procedural Blanks

Procedural blanks are treated identically to samples and carried through the entire extraction procedure. They are used to assess any contamination associated with the extraction and subsequent analysis of samples. At least one field blank or laboratory blank will be analyzed with every 20 field samples.

Initial Calibration and Calibration Checks

Instruments must be calibrated prior to analysis, after each major equipment disruption, or whenever ongoing calibration checks do not meet recommended control limit criteria. All calibration standards used should be traceable to recognized organizations for the preparation of QA/QC materials. Multiple level analyses are performed using several standards containing different concentrations of the analytes of interest to establish the calibration curve. For organic analyses, a 3 to 5 point response factor calibration curve is established that brackets the linear range of analysis. A mid-level standard is then analyzed as a calibration check at the beginning of each analysis and after every 10 samples. For inorganic analyses using the Inductively Coupled Plasma Spectrophotometer (ICP), initial calibration curves are established using ten (for sediment analysis) or eight (for tissue analysis) standards that bracket the linear range of analysis. A curve normalization is run before each set of analyses using an automated procedure that requires adherence to documents QA criteria. A check standard at 1 part per million is then analyzed during the course of the run. For inorganic analyses using the Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS), an initial five-point response factor calibration curve is established that brackets the linear range of analysis. A

mid-level standard is then analyzed as a calibration check.

Matrix Spikes

Matrix spikes are performed to estimate the overall method recovery and the accuracy of chemical analysis. They may also provide information on any interferences which might affect the analysis. Matrix spikes are performed by adding a known concentration of the analytes of interest (the sample should be spiked no more than four times and no less than two times the sample value) to a previously analyzed field sample or laboratory blank. The recovery of spike is then calculated and the percent recovery is calculated as follows.

$$\% R = \frac{S - U}{C_{sa}} \times 100$$

% R	=	percent recovery
S	=	measured concentration in spiked sample
U	=	measured concentration in unspiked sample
C _{sa}	=	actual concentration of spike added

A matrix spike will be run with every 20 field samples, using one of the samples from the batch as a matrix if appropriate.

PRECISION

Precision is defined as the degree to which individual measurements converge upon a single value. Precision is determined by the analysis of laboratory duplicates, which are prepared by homogenizing and sub sampling a sample in the laboratory and carrying the sub-samples through the entire analytical process. Precision will be expressed as the relative percent difference (RPD) for all laboratory duplicates according to the following equation.

$$RPD = \frac{(C_1 - C_2)}{(C_1 + C_2) / 2} \times 100$$

RPD	=	Relative Percent Difference
C1	=	larger of the two observed values
C2	=	smaller of the two observed values

A laboratory duplicate will be analyzed for every 20 field samples.

COMPLETENESS

Completeness is a measure of the amount of valid data obtained from a measurement system compared to the amount that was expected to be obtained under normal conditions. An aspect of completeness that can be expressed for all data types is the percentage of valid data obtained from the measurement process. It also may be expressed as the percentage of samples for which valid data are obtained. Reasonable target completeness values are 90% for samples analysis. The inability to complete a sample analysis is documented in the laboratory notebook with an appropriate explanation.

To ensure that all required samples are analyzed, each sample is assigned a unique identity that is tracked through all stages of an experiment: from assignment of ID to calculation of final concentration(s). The analyst conducting the experiment is responsible for ensuring that 100% of the samples have been completed, or (in the event of sample loss) maintaining records that document the loss. The work assignment manager is responsible for verifying the records of completeness.

REPRESENTATIVENESS

Representativeness expresses the degree to which data accurately and precisely represent a characteristic of a population, parameter variations at a sampling point, a process condition, or an environmental condition. Representativeness will be addressed by the proper handling and storage of samples prior to analysis and analysis in a timely manner so that the material analyzed reflects the original material collected as accurately as possible.

COMPARABILITY

Data comparability depends on the consistency in analytical methodology and the use of standard reporting units for data and reflects the degree to which one data set can be compared to another. Previously demonstrated analytical techniques and the results of laboratory intercomparison exercises will be used to ensure comparability.

DETECTION LIMIT

Organics

Detection limit is the minimum concentration of a substance that can be measured and reported. The Method Detection Limit (MDL) is the minimum concentration of a substance that can be identified, measured, and reported with 99 percent confidence that the analyte concentration is greater than zero. The instrument Limit of Detection (LOD) is the lowest concentration level that can be determined statistically from a blank, while the Instrumental Limit of

Quantitation (ILOQ) is the level above which quantitative results may be obtained. A triplicate analyses of serial dilutions of standard mixtures is performed, and the standard deviation of the difference between the lowest concentration of each analyte instrumentally detectable and a blank values is used to establish the LOD (three times the standard deviation) and LOQ (ten times the standard deviation).

The Method Detection Limits (MDLs) are calculated by spiking a representative sample matrix with target analytes at three to five times the estimated detection limit. Several replicates are analyzed and the standard deviations in analyte concentrations are calculated. MDLs are determined by multiplying the SDs by an appropriate factor.

The Method Limit of Quantification (MLOQ) is the concentration above which quantitative results may be obtained with an assumed degree of confidence. The MLOQ is defined as ten times the MDL. Target MDLs for the analytes of interest are given in Table 2. Typical MLOQ for individual PCBs and OCPs will be in the range of 10-20ng/g; and for individual PAHs, the MLOQ will be about 100-200 ng/g.

Metals

The Method Limit of Quantitation (MLQ) approach is used in the trace metal studies. In this approach the instrument detection limit (IDL) is the lowest concentration level that can be determined with a 99% confidence from replicate analyses of a blank. The Limit of Quantitative Detection (LQD) is defined as 5 times the IDL for ICP analyses, and 10 times the IDL for GFAA analyses. The MLQ is defined as follows: $LQD (ug/L) \times sample\ volume (L) / sample\ dry\ weight (g)$. MLQ values for sediments and biota samples are in units of ug/g. MLQ values for water samples are in units of ug/L. The MLQ is the minimum concentration of a substance that can be measured and reported with confidence as a concentration greater than zero

CORRECTIVE ACTION FOR SAMPLES

The work assignment manager will be notified of any samples that fall outside the established criteria listed in Table 1 and will initiate corrective action which may include review of data calculations, flagging of suspect data, or reanalysis or reparation of individual or an entire batch of samples.

Chemistry Analysis

An assortment of QC check lists shall be prepared by the analyst for each batch of analyses. This check list is an identification tool for any QC problems and can be used to determine if any immediate corrective action is required.

SAMPLE RECEIPT AND STORAGE

Samples received will be handled in such a way to preclude contamination or loss of any of the samples. Sample chain of custody will be maintained by use of a Chain of Custody Record. Recommended sample sizes, containers, preservation techniques and holding times are summarized in Table 2. After receipt and subsampling of samples, all sediment and tissue samples will be held frozen at -20° C until extraction is initiated. Water samples will be preserved by adding methylene chloride and held refrigerated at 1–4° C until extracted. The maximum holding time for any sediment or tissue sample will be 6 months to extraction and 40 days to analysis of the extract. The maximum holding time for any preserved water sample will be 1 week to extraction and 40 days to analysis of the extract.

Sample Archiving

If sufficient sample remains after completing all analyses and subsampling, the remaining sample will be stored frozen at -20° C for a minimum of 6 months after the analyses date.

ANALYTICAL PROCEDURES

Sediment, tissue samples and pore water samples will be chemically analyzed for routine organic and inorganic analytes using the appropriate Standard Operating Procedures (Appendix A). For all analyses, sufficient sample size is necessary to obtain the target MDLs or MLQs listed in Table 3. In cases where there appears not to be enough material for analysis, the Project Officer will be consulted and will determine if it is possible to composite samples to obtain enough sample for a valid analysis.

Sample Analysis Scheme

At least four QC samples are analyzed with every 20 field samples including SRM, blank or matrix spike, field blank and/or laboratory blank and duplicate sample.

REFERENCES

EPA, 1993. Quality Assurance Project Plan, Estuarine Ecological Risk Assessment at the Portsmouth Naval Shipyard, Kittery, Maine, U.S. EPA, Environmental Research Laboratory, Narragansett, RI, 18pp.

Table 1. Key Elements for Quality Control of Chemical Analyses

QC MEASUREMENT	WARNING LIMIT Criteria	CONTROL Limit Criteria	FREQUENCY	CORRECTIVE ACTION (for samples outside the control limit)
1. Initial demonstration of calibration standards. (prior to Analysis of samples)				
A.) Initial Calibration	NA	Organics:<25% for any one analyte, no more than 2 analytes can be >25% Inorganics:<15% for any one analyte	To establish calibration curve prior to sample analysis	NA
B.) Method Detection Limits	NA	NA	At least once each sample matrix	NA
2. Ongoing Demonstration Capability				
A.) Intercalibration Exercises ¹	NA	NA	Two or three per year	NA
B.) Continuing Calibration Checks	NA	Organics:<25% for any one analyte, no more than 2 analytes can be >25% Inorganics:<25% for Mercury, <15% for all others, no more than 2 analytes can exceed	Calibration standards at the beginning, end, and every ten samples	Reestablish initial calibration curve, batch must be reanalyzed
C.) Analysis of Standard Reference Material (SRM) ²	NA	Organics: + / - 30% of the certified concentration range, no more than 30% of the recoveries can exceed the limit with an overall average recovery of 30%. Inorganics: + / -25% diff. for Mercury, 20% for all others with no more than 15% of the recoveries exceeding the limit	1 every 20 field samples	Note in case narrative and flag analytes involved in final data report. Sample batch may be considered for reanalysis

Table 1. Continued

D.) Laboratory Duplicates ³	NA	Organics: <30% RPD; Inorganics: <50% RPD for iron and Aluminum, <30% for all others; no more than 35% of the recoveries can exceed the limit	1 every 20 field samples	All samples associated with duplicates will be noted in the case narrative; batch may be considered for reanalysis based on other QC criteria
E.) Field and Laboratory Blank Analysis	NA	< 20% field sample analyte concentration	1 every 20 field samples	Note affected samples and analytes in the case narrative. Flag correspond- ing data as contaminated.
F.) Matrix Spike ⁴	Recovery should be within the range 50% to 150% for at least 70% of the analytes	NA	1 every 20 field samples	Individual analytes flagged and explained in the case narrative. If more than 30% of the analytes fall outside of the control limit, the batch may be con- sidered for reana- lysis based on other QC criteria.
G.) Internal Standards (Surrogate Internal Standard)	Recovery should be within the range of 40% to 120%	NA	Each Sample	Batch may be considered for reanalysis based on other QC criteria
H.) External Standards (Recovery Standards)	Lab develops its own	NA	Each Sample	NA

NOTES:

1. The purpose of the intercalibration exercise is to assess data comparability. Gross differences between the GSO and other reporting laboratories will be subject to review by the Project Officer to determine if corrective action is necessary for analytes that equal or exceed the MLOQ.
2. Reported results from the analysis of SRMs which are below the MLOQ are not to be used for computing control limits; however those results will be reported in order to identify possible matrix problems and evaluate method performance.
3. Analyte concentrations in the sample duplicate must be above the MLOQ before the RPD can be calculated. It is understood that there will be a higher amount of variability in RPDs calculated for analytes at or near the MLOQ.
4. No control limit is expressed for Matrix Spikes. If analytes fall outside the warning limits, they should be flagged accordingly and explained in the case narrative. The samples should be spiked at a level of not less than 2 times or more than 4 times the sample value, and analytes must be above the MLOQ.

Table 2. Recommended sample sizes, containers, preservation techniques and holding times for sediment, water and biota sample collections.

Media/Analyte	Sample Size (g) ^a	Container ^b	Preservation Technique	Maximum Holding Time ^c	Maximum Extract Holding Time
<u>Sediments</u>					
Particle size	10	P, G	Freeze	6 months	--
Total solids	50	P, G	Freeze	1 year	--
Total organic carbon	50	P, G	Freeze	1 year	--
Petroleum hydrocarbons	100-200	G	Freeze	1 year	--
Acid-volatile sulfides	30	P, G	Freeze	2 months ^e	--
Semivolatile organic compounds	100-200	G	Freeze	1 year	40 days
Pesticides and PCBs	100-200	G	Freeze	1 year	40 days
Mercury	10	P, G	Freeze-dried ^d	2 years ^d	--
Butyltin compounds	100-200	G	Freeze	1 year	40 days
Metals (except mercury)	50	P, G	Freeze	2 years	--
<u>Tissues (whole)</u>					
Semivolatile organic compounds	100	A, G	Freeze	1 year	40 days
Pesticides and PCBs	100	A, G	Freeze	1 year	40 days
Mercury	2	A, G	Freeze-dried ^d	2 years ^d	40 days
Metals (except mercury)	6	A	Freeze	2 years	--

Table 2. con't.

Media/Analyte	Sample Size (g) ^a	Container ^b	Preservation Technique	Maximum Holding Time ^c	Maximum Extract Holding Time
<u>Tissues (after resection)</u>					
Semivolatile organic compounds	25	G, T	Freeze	1 year	40 days
Pesticides and PCBs	25	G, T	Freeze	1 year	40 days
Mercury	2	P, G	Freeze-dried ^d	2 years ^d	--
Metals (except mercury)	6	P, G	Freeze	2 years	--
<u>Pore Water^c</u>					
Metals (except mercury)	25-50	P	Acidify w/ Nitric acid pH < 2.0, cool 4°C	6 months	--
Mercury	10	P	Acidify w/ Nitric acid pH < 2.0, cool 4°C	28 days	

^a Recommended field sample sizes (wet weight) for one laboratory analysis. If additional laboratory analyses required (e.g., replicates), the field sample size should be adjusted accordingly (i.e., multiply the sample size to account for laboratory quality control samples). For tissue samples (after resection), studies using specific org may require more tissue.

^b A - wrapped in aluminum foil
G - glass with Teflon; pre-cleaned jars can be purchased
P - polyethylene
T - PTFE (Teflon)

^c Suggested holding times; no USEPA criteria exist for these variables in these media. The holding time of 1 year semivolatile organic compounds exceeds the USEPA criterion of 14 days; every effort will be made to analyze sample as soon as possible. Sediment and tissue samples may be stored for up to 1 year if $\leq -20^{\circ}\text{C}$. Evaluation Dredged Material Proposed for Discharge in Waters of the U.S. - Testing Manual (Draft) Internal Testing Manual (EPA-823B-94-002) June 1994.

^d Standard reference materials prepared by the U.S. National Institute for Standards and Technology (U.S. NI are freeze-dried and can be stored for at least 2 years. It should, therefore, be acceptable to freeze-dry th samples and hold them for a similar period (Creclius 1994, pers. comm.).

^e Studies (e1, e2) indicate that AVS samples can be stored frozen for up to 10 days with no loss of AVS and may stored frozen up to 5 months with losses of only 10-15% of original values.

1) Analysis of Acid Volatile Sulfide (AVS) and Simultaneously Extracted Metals (SEM) for the Estimation Potential Toxicity in Aquatic Sediments. *Environ. Tox. Chem.*, 12: 1441-1453.

2) Leonard, E.N. et al., 1993. Seasonal variation of acid volatile sulfide core in sediment cores from th northeastern MN lakes. *Hydrobiologia*, v. 271: 87-95.

Table 3. Target Analytes for Chemical Characterization

Analyte	Sample matrix	Target detection limit ^a
Polycyclic Aromatic Hydrocarbons (PAHs)		
	sediment	10 ng/g
	biota	20 ng/g
	water	1.0 µg/L
naphthalene	fluoranthene	
2-methylnaphthalene	pyrene	
1-methylnaphthalene	benz[a] anthracene	
biphenyl	chrysene	
2,6-dimethylnaphthalene	benzo [b] fluoranthene	
acenaphthylene	benzo [k] fluoranthene	
acenaphthene	benzo [e] pyrene	
1,6,7-trimethylnaphthalene	benzo [a] pyrene	
fluorene	perylene	
phenanthrene	indeno [1,2,3-cd] pyrene	
anthracene	dibenz [a,h] anthracene	
1-methylphenanthrene	benzo [ghi] perylene	
Organo-Chlorine Pesticides (OCPs)		
Aldrin	sediment	1 ng/g
hexachlorobenzene	biota	2 ng/g
Mirex	water	0.1 µg/L
o.p' - DDE		
p.p' - DDE		

Table 3. Continued

Analyte	Sample matrix	Target detection limit ^a
Polychlorinated Biphenyl (PCB) Congeners		
8 (2 4')	sediment	1 ng/g
18 (2 2'5)	biota	2 ng/g
28 (2 4 4')	water	0.1 µg/L
52 (2 2'5 5')		
44 (2 2'3 5')		
66 (2 3'4 4')		
101 (2 2'3 5 5')		
118 (2 3'4 4'5)		
153 (2 2'4 4'5 5')		
105 (2 3 3'4 4')		
138 (2 2'3 4 4'5)		
187 (2 2'3 4'5 5'6)		
128 (2 2'3 3'4 4')		
180 (2 2'3 4 4'5 5')		
170 (2 2'3 3'4 4'5)		
195 (2 2'3 3'4 4'5 6)		
206 (2 2'3 3'4 4'5 5'6)		
209 (2 2'3 3'4 4'5 5'6 6')		
Major elements		
aluminum	sediment	10 µg/g
	water, pore water	80 µg/L
	biota	10 µg/g
iron	sediment	15 µg/g
	water, pore water	120 µg/L
	biota	15 µg/g
manganese	sediment	2.0 µg/g
	water, pore water	16 µg/L
	biota	0.2 µg/g

Table 3. Continued

Analyte	Sample matrix	Target detection limit ^a
Trace elements	sediment	0.05-10.0 µg/g
copper	water pore water	0.8-8.0 µg/L
nickel	biota	0.02-0.6 µg/g
chromium		
lead		
silver		
cadmium	sediment	0.05 µg/g
	water, pore water	0.2 µg/L
	biota	0.0125 µg/g
zinc	sediment	2.25 µg/g
	water, pore water	18 µg/L
	biota	2.25 µg/g
arsenic	sediment	1.3 µg/g
	water, pore water	5.0 µg/L
	biota	0.125 µg/g
mercury	sediment	0.025 µg/g
	water, pore water	0.2 µg/L
	biota	0.025µg/g
Butyltins	sediment	1 ng Sn/g
monobutyltin	biota	5 ng Sn/g
dibutyltin		
tributyltin		

^a Sediments and tissues measured on a dry weight basis.

^b Congener number (position of chlorines).

APPENDIX B2

Quality Assurance Project Plan (QAPP)

**Survey of Sediment Toxicity at Navy Education and
Training Center, Newport, RI**

October, 1994

Prepared by:

Science Applications International Corporation
Environmental Testing Center
165 Dean Knauss Drive
Narragansett, Rhode Island 02882

Recommended sample sizes, containers, preservation techniques and holding times for biological samples.

Activity	Sample Matrix	Sample Size	Container Type	Preservation Technique	Storage Condition	Maximum Holding Time	Maximum Extract Holding Time
Chemical Exposure Assessment	Bivalves	10-15g	Ziploc bag	Gut purge live 24 hr, double bag in ziploc, freeze in bag	-20°C/ dark	Analysis dependent	Not applicable
Food Chain Transfer Assessment	Bivalves Lobsters Mummichogs	10-15g	Ziploc bag	Double bag in ziploc, freeze in bag ^k	-20°C/ dark	Analysis dependent	Not applicable
Bulk Sediment Toxicity	Sediment	4 L	Poly-ethylene	Completely Fill and Refrigerate	4±2 °C/ dark/ airtight ^f	14 Days ^f	14 Days ^g
Pore Water Toxicity	Sediment	2 L	Poly-ethylene	Vacuum Extraction ^j	-20°C/ dark/ airtight ^h	7 days ^h	24 hr ⁱ

^fGuidelines to be followed for holding times for samples destined for toxicity testing are as stated in "Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S. - Testing Manual (Draft). EPA-823-B-94-002, June 1994." The document states "Two weeks is recommended; sediments must not be held for longer than 8 weeks prior to biological testing".

^gSediment may be press-sieved at any time during the holding period, but cannot extend the holding period.

^hLong et al., 1994. Magnitude and extent of sediment toxicity in Tampa Bay, Florida. NOAA Tech. Mem. NOS ORCA 78, Silver Spring, MD. 138 pp.

ⁱtime allowed for sample thawing to room temperature immediately prior to toxicity testing.

^jWinger, P.V. and P.J. Lasier. 1991. A Vacuum-Operated Pore-Water Extractor for Estuarine and Freshwater Sediments. Archives of Environmental Contamination and Toxicology. 21:321-324.

^kLobster samples will be resected live for muscle and hepatopancreas prior to freezing. SAIC Standard Operating Procedure: Preparation of Marine Tissues for chemical Analysis SOP 4.01, March, 1995.

1.0 Project Description

The purpose of this project is to provide acute toxicity data for a sediment quality assessment of marine sediments collected in the vicinity of the Navy Education and Training Center in Newport, Rhode Island. Specifically, activities within this project include:

- perform sperm-cell toxicity test on porewater extracted from each sediment,
- perform 10-day solid-phase toxicity tests using the amphipod, *Ampelisca abdita*.

2.0 Technical Design

Specifics of how or where the sediments and porewaters are to be collected are beyond the scope of this QAPP.

3.0 Project Organization and Responsibility

Dr. Glen Thursby, Manager of SAIC's Environmental Testing Center, will have overall responsibility for execution and direction of this project. Dr. Thursby has managed the ETC since it was established in 1990. He has overseen the execution of numerous toxicity testing programs, including four NOAA sediment toxicity surveys in the Hudson-Raritan Estuary, Long Island Sound, Tampa Bay, and Boston Harbor, and our past toxicity testing for EPA's EMAP-Virginian Province. Dr. Thursby will be the primary interface with on any subject related to the performance of this work. He will provide quality assurance review and he will directly support the data analysis and interpretation phases of this project. Dr. Thursby's authority and responsibilities include:

1. Overseeing the scheduling and management of efforts of assigned personnel;
2. Ensuring that products meet and SAIC standards, including those related to Quality Assurance;
3. Determining when all requirements of the tasks have been satisfactorily completed; and
4. Insuring that budgetary requirements of the tasks are adhered to.

Ms. Cornelia Mueller will direct all the toxicity testing at SAIC's Environmental Testing Center. She will be responsible for assuring that the testing is conducted according to approved standard operating procedures, and will monitor the financial status, and report technical and financial progress to the Project Manager.

4.0 Sample Custody

When samples arrive at the laboratory shipments are checked against the accompanying chain-of-custody form to verify any sample-specific information. Custody is transferred either to the Laboratory Manager or her assistant. Copies of the original chain-of-custody form are sent to the client as verification of receipt. Sample storage, date, and type of manipulation/analysis also will be recorded as the samples progress through the laboratory. All samples will be stored refrigerated at 4 °C.

5.0 Methods

5.1 Source of Test Animals

Ampelisca abdita will be collected from tidal flats in the Pettaquamscutt (Narrow) River. Surface sediments (8 to 10 cm) will be collected with a pool scoop and sieved through a 0.5-mm screen. Amphipods floating on the air/water interface will be transferred with a small aquarium dip net and transported to the laboratory in site water. At the laboratory, amphipods will be transferred to holding containers that contain 4 L of filtered seawater (28 to 30 ppt) over a 2 to 4 cm layer of pre-sieved collection site sediment. Amphipod density will not exceed 400 amphipods per holding container. Fifty percent of the water will be replaced every other day when the amphipods are fed an abundance of the diatom *Phaeodactylum tricornutum*.

Arbacia punctulata adults are maintained year-round at the ETC at approximately 15 °C. The sea urchins are fed freshly collected kelp (*Laminaria* spp.) , *ad libitum*.

5.2 Amphipod Toxicity Test Procedures

Standard Operating Procedures are attached for the 10-day, solid-phase with *Ampelisca abdita*. Amphipods will be exposed to test sediments for 10 days with five replicates under static conditions using 30 ppt filtered seawater. Exposure chambers will consist of quart-sized canning jars with an inverted glass dish as a cover. Two hundred milliliters of control or test sediment will be placed in the bottom of the jar and covered with approximately 600 ml of water. Air will be delivered via air pumps into the water column by a polystyrene 1-ml pipette inserted through a hole in the cover to ensure acceptable dissolved oxygen concentrations (>60% saturation). To stabilize the temperature, tests will be conducted in a water bath and lighting will be continuous during the 10-day test to inhibit swimming behavior of the organisms.

At the beginning of each test, twenty animals for each species will be distributed randomly into 100-ml plastic beakers containing the appropriate water for that species. The beakers will be examined for dead or outsized animals, which will be replaced. The beakers will be randomized, air delivery to the exposure containers stopped, and the amphipods and mysids added to their respective test chambers. For amphipods, after one hour, nonburrowing animals will be replaced, and air delivery will be restarted, initiating the test. *Ampelisca abdita* will not be fed during the test.

All observations conducted during the test and at completion will be performed "blind" to ensure that observer bias will not effect results. Exposure containers will be checked daily and the number of individuals that are dead, moribund, on the sediment surface, and on the water surface will be recorded. Salinity (refractometer), dissolved oxygen (Orion model 970800), and pH (Orion model 250A) will be monitored twice during the test (generally on days 2 and 7).

The primary response criteria to indicate toxicity of test sediments in these assays will be survival. After 10 days, the assay will be terminated and the contents of each exposure chamber sieved through a 0.5-mm mesh screen. The material retained on the sieve will be sorted under a stereomicroscope and the recovered animals counted. Any missing individuals will be assumed to have died and decomposed during the test and will be counted as dead. "Live-picking" of the samples at the termination of the tests will be the preferred method of analysis. However, if problems develop in the laboratory, and live-picking will not be possible, the sample material will be sieved and preserved in 5% buffered formalin with Rose Bengal stain for later examination. Statistical significance will be calculated for each sample with a mean survival less than that of its respective performance control. Survival data from these samples will be compared to that of the control with a one-way, un-paired, *t*-test that assumes unequal variance. The alpha level will be 5%.

5.3 Sperm-Cell Test Procedures

Test procedures will follow EPA's protocol for complex effluents (EPA, 1988--Standard Operating Procedure attached). Female sea urchins will be transferred to a large Carolina dish filled with enough seawater to just cover their shells. Eggs will be obtained from the females by electrical stimulation. The electrodes from a 12-volt transformer will be gently placed on the shell close to the gonadopores for about 30 seconds. The red eggs, which pool above the gonadopores, will be collected with a 10 ml disposable syringe with a blunted large-gauge needle. Eggs will be collected from all females and kept at room temperature (about 20 °C) until use (no longer than two hours). The final egg concentration in the stock solution will be 2,000 per ml. Sperm also will be collected from male sea urchins by electostimulation, using a 2 ml syringe, and kept on ice. The time of sperm collection will be recorded; the sperm will be exposed to the test material within one hour after collection. The sperm will be diluted to a stock concentration of 50 million per ml, and the stock kept on ice until use.

A test begins with the introduction of 100 µl of well-mixed sperm stock to each test and control vial (which contained 5 ml of sample or control seawater) so that there will be about 5 million sperm in each vial. The test vials then will be covered, the time will be recorded and the vials held at 20 to 22 °C for one hour. Both natural seawater and brine controls will be used when the samples required salinity adjustments. After one hour, the egg stock will be mixed by gentle aeration and 1 ml of stock added to each exposure vial. The vials will be gently swirled to insure mixing and covered again. After an additional 20 minutes, 1 ml of 10% buffered formalin in seawater will be added, terminating the test. The fertilization will be evaluated by examining 100 individual embryos from each

replicate with a compound microscope at 100X magnification. A reference toxicant test will be performed using sodium dodecyl sulfate. Statistical significance will be calculated for each sample with a mean fertilization rate less than that of the performance control. Fertilization data from these samples will be compared to that of the control with a one-way, un-paired, *t*-test that assumes unequal variance. The alpha level will be 5%.

6.0 Quality Assurance

SAIC has extensive checks and balances in place to ensure the integrity of the data that is generated for any project. Copies of all original raw data sheets are kept in chronological order in notebooks separate from the working copies. The working copies are kept in a project specific file along with copies of all other ancillary information, such as chain-of-custody forms. All data reported will be checked for error in transcription, calculation or computer input by a minimum of two individuals.

Each sediment sample will be logged into the Environmental Testing Center on a standardized form at the time of arrival and assigned a sample number. This sample number is used to track a sample from arrival, through testing, to disposal. The information on the log-in sheet indicates proper handling within the laboratory, which toxicity tests are to be performed, as well as maximum holding time. Arrival and collection dates also are recorded on the log-in sheet and are used to calculate actual holding time prior to testing. Sample and control sediments will be held at 4 °C before testing.

The quality of amphipods used in toxicity testing is assessed prior to the beginning of a test and throughout the entire test. During acclimation and holding, the general health of the organisms is monitored daily and if total mortality exceeds acceptable limits (10%), then these organisms are not used for toxicity testing. Daily records are kept of feedings, temperature, salinity and any other critical factors needed to assure the acceptability of the organisms before use in toxicity testing. During a test, the quality of organisms used will be monitored through the use of a control treatment and reference toxicant test (SDS). The control treatment consists of exposing the test organisms to clean sediment under the same conditions that the treatments are exposed. Survival of organisms overall should be greater than or equal to 90% for *A. abdita* in the control sediment. Acceptance of control survivals less than the desired limits will be dependent on the overall performance of an entire test series and each sample is considered for re-testing independent of the others. If mean survival of a sample is greater than 80%, then that sample is generally not considered for re-testing.

Proper overlying water quality and other conditions necessary to the survival of the organisms will be maintained and documented. Chamber temperature will be recorded daily. Waterbath temperature will be monitored continuously. The highest and lowest temperature during a 10-day test should not differ by more than 2 °C. Dissolved oxygen, salinity, and pH will be measured twice during the test. Aeration of each test chamber will be maintained such that dissolved oxygen concentration is generally >90% saturation. Any chambers that have a dissolved oxygen concentration <60% saturation will be

considered unacceptable. Salinity should not vary by more than 1 ppt and pH by more than 0.5 within a group of replicates. Variations in any of these parameters that exceed these limits will be reported along with documentation of the appropriate corrective action.

For sea urchins, records are kept of feedings, temperature, salinity, and any other factors needed to assure the acceptability of the organisms before use in toxicity testing, including ability to spawn. During a test, the quality of the gametes used will be monitored through the use of a control treatment and reference toxicant test (SDS). The control treatment consists of exposing the gametes to clean natural seawater under the same conditions that the pore waters are exposed. Fertilization in the control seawater should be at least 70%. If fertilization in the control treatment is unacceptable, then the porewater samples will be re-tested. If salinity of the pore water is less than 30 ppt, then the salinity may be adjusted to 30 ppt using hypersaline brine. If this occurs, an additional control consisting of diluted brine will be added.

7.0 Calibration Procedures/Preventative Maintenance

A variety of physical measurements are made during each solid-phase toxicity test procedure. These include pH, salinity, dissolved oxygen, and temperature. The method used, the level of precision, and the frequency and method of calibration are listed in appropriate SOPs. The frequency of measurement is test specific, as is the type of biological data monitored, and also is detailed in the SOPs for the various tests.

The refractometer and dissolved oxygen and pH meters are calibrated to standard preparations as recommended by the manufacturer. The refractometer calibration is checked at least yearly with standard seawater and with distilled water before each day's use. The dissolved oxygen and pH meters are calibrated before each use. All prior-to-use calibrations are documented on the data sheets for a given toxicity test. All routine calibrations such as the refractometer seawater check, the checking of the balance with calibration weights and semi-annual checks of all thermometers against a factory calibrated thermometer are documented on standardized calibration sheets. Originals are archived by instrument in a notebook.

8.0 Audits

Because SAIC maintains personnel dedicated solely to QA, internal audits can be arranged to monitor the implementation of the ETC's QA plan. The ETC welcomes and is prepared for external audits performed by clients at any point during a particular project. Any deficiencies noted during an audit will be documented and corrected.

9.0 Data Validation and Documentation

All toxicity data reported are checked for error in transcription, calculation, or computer input by a minimum of two individuals. First, the individual who enters the original data checks for errors and signs a copy of the data outputs. Second, a different individual

rechecks 100% of the data and countersigns the data sheets. All sample logs and data forms are reviewed to ensure that requirements for sediment holding and integrity, data quality assessment, and equipment calibration have been met. Data that do not meet these requirements are reported with an accompanying explanation of the problems encountered. After the raw data passes the above QA checks, a copy of the computer file is used to perform all statistical analyses. A summary data file is created with the summary information from each toxicity test series, including mean, standard deviation, and the results of the statistical comparisons. When all toxicity test series have been completed, a master summary file is created by merging the test series summary files. No manual data entry occurs after the initial entry of the raw data.

At least two copies of the raw data sheets are kept, the original in a master notebook and a photocopy in the working file. All computer files associated with a project are kept in a project sub-directory on the Laboratory Managers computer. Backup copies on diskette also are kept in the Project Manager's office. The Laboratory Manager or the Project Manager will maintain a record of which computer files are the most up to date.

10.0 Environmental Compliance and Health and Safety

Ms. Lorraine Wright is SAIC's on-site Environmental Compliance and Health and Safety (ECHS) Officer. Ms. Wright reports directly to SAIC's corporate ECHS Officer, Mr. Gary Waggoner, in all matters pertaining to proper procedures and conditions. SAIC complies with all State and Federal laws pertaining to environmental compliance and health and safety.

10.1 Logging in Samples

All samples are assumed to be toxic unless proven otherwise. Therefore, gloves, safety glasses, and a laboratory coat are put on before opening packages containing environmental samples.

10.2 Sediment Handling

All sediment handling (press sieving, placing sediments in exposure containers, and sieving at test breakdown) is performed under a certified hood. Protective gear is at a maximum when press sieving. Safety glasses, face shield with chin guard, poly laminated coveralls and apron, dielectric boots, and three pairs of gloves (latex, Silvershield, and 18" nitrile).

10.3 Checking Solid-Phase Tests

At a minimum, safety glasses, white tyvek coats, and latex gloves are worn to check tests of samples of unknown hazard.

10.4 Sediment Disposal

Personnel disposing of waste sediment into a drum must wear the appropriate garb. This

includes a total-body poly laminated coveralls and apron, three pairs of gloves (18" nitrile over Silvershield over latex), full face shield with chin guard, and poly laminated booties over personal shoes or dielectric boots. Extra precaution and every effort is taken to ensure minimum spillage. A thorough cleaning is performed directly following dumping of sediment. Alconox, hot water, and paper towels are used until the site is free of visible stray sediment. Used paper towels are disposed of directly in the dumpster. A TCLP chemical analysis is conducted on the drum of sediment by an independent, outside contractor. Once it passes, the sediment is sent to Central Land Fill in Johnston, Rhode Island, with the land fill's permission.

10.5 Spills

Spilled sediment is not allowed to dry. Small spills are wiped up with paper towels which are taken directly to the dumpster before they dry out. For larger spills, sediments are scooped up and placed directly into a designated sediment waste drum. The remaining residue is removed with paper towels.

10.6 Miscellaneous Measures

Lab coats and other protective equipment are not worn outside the laboratory. Red-labeled pens, scissors, etc., are not brought outside the laboratory area. There is no disk washing in laboratory sinks. There is no eating or drinking in the laboratory. No food or beverages may be stored in the refrigerators designated for samples. Hands are washed with Chlorostat antiseptic soap before leaving laboratory area after samples have been handled.

**ENVIRONMENTAL TESTING CENTER
STANDARD OPERATING PROCEDURE**

FERTILIZATION TEST USING THE SEA URCHIN *ARBACIA PUNCTULATA*

1. OBJECTIVE

- 1.1 This document describes the methods used to conduct the Sea Urchin Sperm Cell Fertilization Test.
- 1.2 This method measures the toxicity of effluent and receiving waters to the gametes of the sea urchin *Arbacia punctulata* during a 1 hr and 20 min. exposure. The purpose of the sperm cell toxicity test is to determine the concentration of a test substance that reduces fertilization of exposed gametes relative to that of the control.
- 1.3 are required to complete this test.

2. SAFETY

- 2.1 Environmental samples may contain hazardous biological or chemical constituents. Proper attire should be worn.
- 2.2 Fertilized eggs preserved in formalin should be examined under a fume hood.

3. MATERIALS

- 3.1 Air pump.
- 3.2 Plastic 1 ml pipets.
- 3.3 Centrifuge, bench top, variable speed.
- 3.4 Fume hood.
- 3.5 Dissecting scope.
- 3.6 Compound scope.
- 3.7 Sedgwick-Rafter counting chamber.
- 3.8 Hemacytometer.
- 3.9 Count register, 2-place.
- 3.10 Ice bucket.
- 3.11 Centrifuge tubes, conical, 50 ml. plastic.
- 3.12 18 oz, tall, glass jar.
- 3.13 Two large dishes.
- 3.14 Wash bottles filled with deionized water and natural sea water.
- 3.15 Transformer, 10-12 Volt, with steel electrodes.
- 3.16 Two syringes: 1 ml, and 10 ml, with 18 gauge, blunt-tipped needles (tips cut off).
- 3.17 5 ml, automatic pipet.
- 3.18 1 ml, adjustable pipet.
- 3.19 Permanent marker.
- 3.20 Sea urchins, 4 or 5 of each sex.
- 3.21 Scintillation vials, 20 ml. disposable.
- 3.22 Parafilm.
- 3.23 Formalin, 2.5% buffered in sea water, filtered.

1. Add 10 ml 10% acetic acid in seawater to Vial C. Cap Vial C and mix by inversion.
 2. Add 1 ml of killed sperm from Vial C to 4 ml seawater in Vial E. Mix by gentle inversion.
- 4.5.11 Add sperm from Vial E to both sides of the Neubauer hemacytometer.
- 4.5.12 Let the sperm settle for 15 min.
- 4.5.13 Count the number of sperm in the central 400 squares on both sides of the hemacytometer using a compound microscope (400X).
- 4.5.14 Average the counts from the two sides.
1. SPM in Vial E = 104 x average count from Vial E.
- 4.5.15 Calculate the SPM in all other suspensions using the SPM in Vial E.
1. SPM in Vial A = 40 x SPM in Vial E.
 2. SPM in Vial B = 20 x SPM in Vial E.
 3. SPM in Vial D = 5 x SPM in Vial E.
 4. SPM in original sperm sample = 2000 x SPM in Vial E.
- 4.5.16 Choose the vial containing a sperm concentration greater than 5×10^7 SPM and dilute to 5×10^7 SPM.
1. Actual SPM / (5×10^7) = dilution factor (DF).
 2. [(DF) x 10] - 10 = ml of seawater to add to vial.
- 4.6 Prepare the eggs.
- 4.6.1 Using a tabletop centrifuge, wash the pooled eggs twice with control seawater.
- 4.6.2 Spin for three minutes at lowest possible setting.
- 4.6.3 If the wash water becomes red, the eggs have lysed and must be discarded.
- 4.6.4 Dilute the egg stock to 2000 ± 200 eggs/ml using control seawater.
- 4.6.5 Remove the final wash water and transfer the washed eggs from the centrifuge tube to a beaker containing a small volume (about 50 ml) of control water by pouring off the wash water and refilling the tube with control water.
- 4.6.6 Gently invert the tube to suspend the eggs.
- 4.6.7 Add more control water to the beaker to bring the egg stock to a volume of 200 ml.
- 4.6.8 Mix the egg stock using gentle aeration.
- 4.6.9 Cut the point from a 1 ml pipet tip.
- 4.6.10 Transfer 1 ml of eggs from the egg stock to two vials containing 9 ml of control water.
NOTE: these vials contain an egg suspension diluted 1:10 from the egg stock.
- 4.6.11 Mix the contents of the vial by inversion.
- 4.6.12 Cut the point from a 1 ml pipet tip.
- 4.6.13 Transfer 1 ml of eggs from the vial to a Sedgwick-Rafter counting chamber.
- 4.6.14 Count all eggs in the chamber using a dissecting microscope.
- 4.6.15 Average the numbers obtained from both vials to get the "egg count".
- 4.6.16 Calculate the concentration of eggs in the stock. Eggs/ml = 10 x (egg count).
- 4.6.17 Dilute the egg stock to 2000 eggs/ml.
1. If the egg count is equal to or greater than 200: (egg count) - 200 = volume (ml) of control water to add to egg stock.

Project: _____

Experiment # _____

Start Date: _____

Time Table: _____ Fertilization or Cleavage

- Sperm Collection: _____
- Sperm Added: _____
- Egg Collection: _____
- Eggs Added: _____
- Fixitive Added: _____
- Samples Read: _____

Sperm Dilutions

Hemocytometer Count of vial E:

Side 1 _____

Side 2 _____

Average _____ x 1.00E + 04 = _____ Sperm per ml (SPM) in vial E

SPM 'E' x 40 = _____ (SPM vial 'A')

SPM 'E' x 20 = _____ (SPM vial 'B')

SPM 'E' x 5 = _____ (SPM vial 'D')

Vial Selected for dilution: _____

Dilution Factor = Concentration of chosen vial / 5.00E + 07

Dilution Factor = _____

Volume of Seawater to add = (Dilution Factor x 10) - 10

Volume = _____ mls

Egg Dilution:

Sedgwick-Rafter count: _____ 1:10 dilution of egg stock

Egg Count 1 _____

Egg Count 2 _____

Average _____ Egg Count

Dilution of Egg Stock = Egg Count - 200

Vol. seawater to add to egg stock = _____

QA egg stock dilution

Egg Count 3 _____ x 10 = egg/ml in final egg stock

Final Egg Stock Concentration = _____

Second Sedgwick-Rafter Count (if necessary):

Egg Count 1 _____

Egg Count 2 _____

Average _____ Egg Count

Dilution of Egg Stock = Egg Count - 200

Vol. seawater to add to egg stock = _____ mls

QA egg stock dilution

Egg Count 3 _____ x 10 = egg/ml in final egg stock

Final Egg Stock Concentration = _____

Comments: _____

Test Performed by: _____

ENVIRONMENTAL TESTING CENTER STANDARD OPERATING PROCEDURE

AMPELISCA COLLECTION

1. OBJECTIVE

- 1.1 The purpose of the document is to describe the methods used to collect *Ampelisca abdita*.
- 1.2 *Ampelisca* are used 10-day solid-phase tests to determine sediment toxicity.
- 1.3 Eight hours are required to complete these methods.

2. SAFETY

- 2.1 Canoes and buckets full of sediment are heavy! Keep your back straight and use your leg muscles while lifting.
- 2.2 The launch site is rocky; care must be taken to avoid tripping.
- 2.3 Life jackets must be worn while canoeing to and from the collection site.

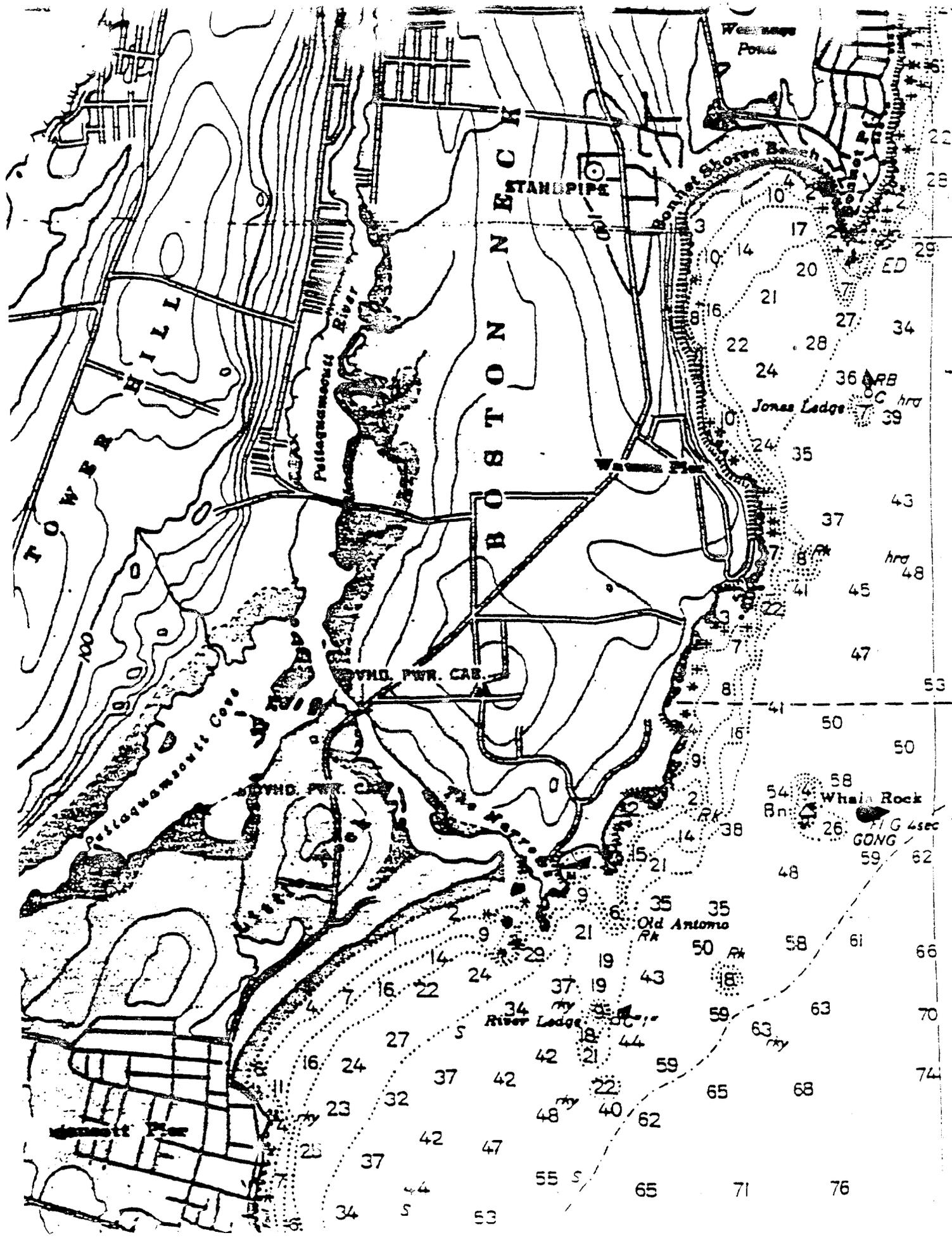
3. MATERIALS

- 3.1 One pair of waders per person.
- 3.2 One pair of 'arms' (shoulder-length gloves) per person.
- 3.3 One life vest per person.
- 3.4 One 12" diameter 0.5 mm field (with handles) sieve per person.
- 3.5 Pool scoop (optional).
- 3.6 One green bucket with lid per 1000 animals needed.
- 3.7 Field thermometer.
- 3.8 Vial labelled with date.
- 3.9 Canoe.
- 3.10 Paddles (include a spare).
- 3.11 Anchor (optional).
- 3.12 First aid kit.
- 3.13 Sunscreen.
- 3.14 Drinking water.
- 3.15 Truck.
- 3.16 Rope.
- 3.17 Short rubber gloves for winter paddling.
- 3.18 Appropriate personal clothing-weather dependent.
- 3.19 Data Sheet AMP-011 "*Ampelisca abdita*, Field Collection and Holding" (attached).

4. METHOD

- 4.1 The launch site is reached by turning down the small access road on the northwest (Saunderstown) side of the bridge on Route 1A.
- 4.2 Unload the truck.
- 4.3 Load the canoe with all field gear, and chain the first aid kit to the bow.
- 4.4 Pull waders to hips, but **do not** pull the straps up.

- 4.5 Put life vest on.
- 4.6 Paddle out to the collection site (indicated on map).
- 4.7 Look for an abundance of tubes on the bottom (if available, a glass bottom bucket may be helpful).
- 4.8 Pull wader straps up over shoulders and put on 'arms' (long gloves).
- 4.9 Life vest can be removed at this time.
- 4.10 Carefully get out of the canoe and anchor it. (Or tie it to a paddle stuck in the mud.)
- 4.11 Take the water temperature (near the bottom) and collect a water sample in the vial.
- 4.12 Collect *Ampelisca*.
- 4.12.1 Using the sieve or pool scoop, gently scrape the top layer of tubes (approx. 1-2") from the bottom. Do not scoop too deep. If using the pool scoop, transfer the mud to a sieve. At high tide, this operation can be done from the canoe.
- 4.12.2 Vigorously shake and slap sieve up and down against the water surface so that most of the sediment passes through, while the tubes remain on the sieve.
- 4.12.3 Gently glide the sieve along the water surface to force the material to one side of the sieve.
- 4.12.4 Gently lower the 0.5 mm sieve into the water without completely submerging it so that amphipods float, estimate the number of amphipods.
- 4.12.5 Deposit the material into the covered bucket.
- 4.12.6 Keep count of your sieves.
- 4.12.7 When enough animals are collected, make sure there is some overlying water in the buckets.
- 4.12.8 Keep the lids on the buckets at all times.
- 4.13 Carefully climb back into the canoe, remove the 'arms', and pull down the straps of your waders and put on your life vest.
- 4.14 Paddle back to the truck, unload and secure the canoe and gear.
- 4.15 Hang a red bandana or cloth on the outboard end of the canoe.
- 4.16 Drive to the ARK and aerate the buckets by connecting silastic lines to aquarium pumps.
- 4.17 Process animals (see ETC SOP).
- 4.18 Rinse truck and all other equipment with tap water.
- 4.19 Hang waders to dry.
- 4.20 Fill out all necessary data on Data Sheet AMP-011.



**ENVIRONMENTAL TESTING CENTER
STANDARD OPERATING PROCEDURE**

LABORATORY PROCESSING OF FIELD COLLECTED *AMPELISCA*

OBJECTIVE

- 1.1 The purpose of this document is to describe the procedures used to process *Ampelisca abdita* for holding and acclimating in the laboratory before testing.
- 1.2 *Ampelisca* are held in the laboratory to acclimate ambient temperature and field conditions to test temperature and laboratory conditions.
- 1.3 Four hours are required to complete these procedures.

SAFETY

- 2.1 Wear dielectric boots at the ARK because electric air pumps will be plugged in nearby.
- 2.2 Buckets full of sediment are heavy! Keep your back straight and use your leg muscles when lifting.

MATERIALS

- 3.1 Large (32" diameter) 0.5 mm sieve - optional.
- 3.2 Large (32" diameter) 2.0 mm sieve - optional.
- 3.3 Sizing sieves (12" diameter): 1.7 mm, 1.0 mm, .71 mm.
- 3.4 50 mm aquarium fish nets - one per person.
- 3.5 Four large dishes (or similar).
- 3.6 Specimen cups.
- 3.7 One for each holding jar (plus extras for gravids, dead, etc.).
- 3.8 Squeeze bottle containing seawater - one per person
- 3.9 Wide bore pipettes with bulbs - one per person.
- 3.10 Holding jars (see ETC SOP).
- 3.11 One air pump with tubing and pipets.
- 3.13 Data Sheet AMP-021 "Field Collection and Holding" (attached).

METHODS

4. Sieve out field collected *Ampelisca*.
 - 4.1.1 Rinse and half-fill dishes with ambient temperature seawater.
 - 4.1.2 Spray ambient temperature seawater into field collection bucket to loosen the amphipods from the sediment.
 - 4.1.3 Skim the amphipods from the surface with an aquarium net and transfer them to a Carolina dish.
 - 4.1.4 Continue until no more pods can be flushed to the surface. If this operation alone produces sufficient pods, proceed to 3.0 and skip the sieving sequence.
 - 4.1.5 Pour half of a bucket's contents (tubes and sediment) onto large 2.0 mm sieve which is inserted into the large 0.5 mm sieve. (Sieves are set up over a settling bin to catch sediment)

- 4.1.6 Loosen the amphipods from their tubes by spraying the material on the sieve with moderate force generated by pinching the end of the hose.
- 4.1.7 Remove the 2.0 mm sieve when all the material has been sprayed thoroughly.
- 4.1.8 Vigorously shake and slap the 0.5 mm sieve up and down (works best with 2 people).
- 4.1.9 Gently lower the 0.5 mm sieve into the water without completely submerging it so that amphipods float.
- 4.1.10 Skim the amphipods from the surface of the water within the sieve using an aquarium net and transfer them to a dish.
- 4.1.11 Aerate the dish with an aquarium pump.
- 4.1.12 Repeat the shaking and skimming process until only a few amphipods remain in the sieve.
- 4.1.13 Pour more material into the sieve from the field bucket and repeat the above process; occasionally cleaning debris and tubes out from the 2.0 mm sieve.
- 4.2 *Size Ampelisca*
 - 4.2.1 Rinse and half-fill three dishes.
 - 4.2.2 Label the dishes; >0.5 , <0.71 , >0.71 , <1.0 , >1.0 , <1.73 .
 - 4.2.3 Stack the sieves in ascending order (0.5 mm on bottom, .71 mm, 1.0mm, and 1.7 mm on top).
 - 4.2.4 Pour collected amphipods slowly onto the 1.7 mm sieve
 - 4.2.5 Thoroughly, but gently, rinse the amphipods through the stacked sieves to separate them by size.
 - 4.2.6 Using a squeeze bottle, rinse each sieve into the appropriate dish.
 - 4.2.7 If there is too much other material in the sieve, sink it nearly to the rim in the tank, allow the crud to settle, then skim off the animals with a net.
 - 4.2.8 Place each size in a separate dish.
- 4.3 *Counting out Ampelisca.*
 - 4.3.1 Take out as many specimen cups as you have holding jars.
 - 4.3.2 Rinse and half-fill specimen cups with seawater.
 - 4.3.3 Randomly pipette 10 medium size amphipods (> 1.0 , < 1.7) into each specimen cup until each cup contains a maximum of 350 amphipods.]
 - 4.3.4 If there are not enough medium size amphipods, use the small (> 0.71 , < 1.0) amphipods. These amphipods can be added until 350 is reached. (Note that at some times of the year we may need to segregate holding jars by size.
 - 4.3.5 Entire jars should contain the same size animals. Note size on cups.
 - 4.3.6 Record the number of animals/holding jar on the Data Sheet AMP-021, along with the sizes used.
- 4.4 Adding the amphipods to the holding jars (see ETC SOP).
- 4.5 Turn off the air leading to the holding jars and gently remove approximately 150 ml of water from each jar.
- 4.6 Empty one specimen cup of amphipods into each holding jar; swirling cup to break up any clumps of amphipods.
- 4.7 Push amphipods stuck on the air-water interface down with (CLEAN) fingers or a pipet.
- 4.8 Wait a few minutes for the amphipods to swim down to the sediment surface then turn the air to the jars back on.
- 4.9 Clean all equipment with fresh water.

**ENVIRONMENTAL TESTING CENTER
STANDARD OPERATING PROCEDURE**

HOLDING OF *AMPELISCA* PRIOR TO TESTING (STATIC-RENEWAL)

1. OBJECTIVE

- 1.1 The purpose of this document is to describe the methods used to hold *Ampelisca* in the laboratory before testing.
- 1.2 *Ampelisca* is held in the laboratory for acclimation and observation purposes.
- 1.3 Four hours are required to complete these procedures.

2. SAFETY

- 2.1 No specific safety precautions are required.

3. MATERIALS

- 3.1 Data Sheet AMP-031 "Field Collection and Holding" (attached).

4. METHODS

- 4.1 Set up holding jars 24 hours before animals are collected in a controlled temperature culture box or waterbath, and aerated. The initial temperature of the seawater in the holding jars is no more than 3°C higher than the field temperature.
- 4.2 Place approximately 4 cm of collection site sediment into holding jar.
- 4.3 Rinse down sides with seawater to remove excess sediment from the sides.
- 4.4 Renew the holding jars before the animals are added.
- 4.5 Add amphipods to the holding jars (see ETC SOP).
- 4.6 Place airline with attached pipet into holding jar so that it is approximately 6 cm below the water surface. Use gentle aeration.
- 4.7 Seawater renewal and feeding (seawater renewal should take place in the morning and feeding in the afternoon)
 - 4.7.1 Remove airlines.
 - 4.7.2 Check for any emerged amphipods; gently prod down to encourage burrowing.
 - 4.7.3 Siphon off approximately 75% of water volume (renewal) or 1L (feeding).
 - 4.7.4 Refill with clean seawater (for renewal) or 1 liter of diatom culture, *Phaeodactylum*, and clean seawater (for feeding), using a turbidity reducer so as not to disturb tubes or sediment. Feeding and renewal can be combined if necessary.
 - 4.7.5 Replace airlines.
- 4.8 Cover holding jars to simulate night time conditions and enhance feeding.
- 4.9 Monitor temperature daily and record on Data Sheet AMP-031.
- 4.10 If amphipods require temperature acclimation to 20°C, the temperature is increased or decreased by 3°C per day and recorded on Data Sheet AMP-031. Temperature can be increased no more than 3°C per day and animals should be maintained at 20°C for at least 48 hr prior to use in test.

**ENVIRONMENTAL TESTING CENTER
STANDARD OPERATING PROCEDURE**

PRESS SIEVING SEDIMENT FOR TOXICITY TESTS

1. OBJECTIVE

- 1.1 The purpose of this document is to describe the methods used to press sieve sediment for use in toxicity tests.
- 1.2 Sediment samples are press sieved through a 2.0 mm sieve in order to remove large debris or predators. If a sample contains amphipods, the sample is press sieved first through a 2.0 mm and then a 1.0 mm sieve in an attempt to remove the resident amphipods.
- 1.3 Eight hours are required to complete these procedures.

2. SAFETY

- 2.1 Sediment and water samples should always be regarded as hazardous. They may contain toxins and/or infectious agents. Press sieving must be conducted in, or in front of a hood.
- 2.2 Protective clothing to be worn include safety glasses, face shield, latex gloves, silvershield gloves nitrile gloves, waterproof blue lab coat or poly laminated coveralls, poly lam apron, and dielectric boots.

3. MATERIALS

- 3.1 12", 2.0 mm sieve.
- 3.2 12", 1.0 mm sieve.
- 3.3 Round plastic bin.
- 3.4 Plexiglas paddle.
- 3.5 Nylon spoon and spatula.
- 3.6 Seawater for rinsing sieves, bin, etc.
- 3.7 Data Sheet AMP-041 "10-Day Solid-Phase -- Randomization Sheet" (attached).
- 3.8 Data Sheet AMP-042 "Sample Log for Walk-In Refrigerator #2" (attached).
- 3.9 Data Sheet AMP-043 "Waste Log for Barrel Number X" (attached).

4. MATERIALS

- 4.1 Pour, or scoop with nylon spoon, entire contents of sample container into sieve. **DO NOT ADD ANY WATER!**
- 4.2 Push sediment through 2.0 sieve with Plexiglass paddle or nylon spoon. If sample contains resident amphipods stack the 2.0 mm sieve on top of the 1.0 mm sieve and press sediment through both sieves.
- 4.3 Rinse out sample container with seawater; discard rinse into settling bucket.
- 4.4 Homogenize sieved sediment by stirring and return to original container for storage or add to test chambers (see ETC SOP).
- 4.5 Label sample container "PRESS SIEVED", and date of sieving.
- 4.6 Record sample information on Data Sheet AMP-041.
- 4.7 If samples are to be stored until testing complete Data Sheet AMP-042.

- 4.8 Between samples, rinse all equipment into settling bucket using seawater.
- 4.9 After the last sample has been sieved rinse all equipment with tap water and then with DI water.
- 4.10 Discard coats and coveralls.
- 4.11 Rinse face shields, silvershield and nitrile gloves. These gloves may be used for about 8 hrs. before discarding.
- 4.12 Allow the waste sediment to settle overnight then decant the overlying water down the drain.
- 4.13 Scoop the sediment out with a designated waste scoop and add to the appropriate 55 gal waste drum. Add the necessary information to Data Sheet AMP-043.

10 Day Solid Phase Test--Randomization Sheet

Project: _____
Species: _____

Experiment #: _____

Jar #	Sort	Client #/Descriptor	Carboy #	Sample Description / Sample QA
	A1	LIS Control		Sign: CSign: /Sign: Csign:
	A2	LIS Control		Sed Type: sand mud clay
	A3	Jar #:		Odor/Color: fishy sulfur oily fecal / brown gray black
	A4	LIS Control		Other Notes: PODS
	A5	LIS Control		Press Sieved: 2mm 1mm
	B1			Sign: CSign: /Sign: Csign:
	B2			Sed Type: sand mud clay
	B3			Odor/Color: fishy sulfur oily fecal / brown gray black
	B4			Other Notes: PODS
	B5			Press Sieved: 2mm 1mm
	C1			Sign: CSign: /Sign: Csign:
	C2			Sed Type: sand mud clay
	C3			Odor/Color: fishy sulfur oily fecal / brown gray black
	C4			Other Notes: PODS
	C5			Press Sieved: 2mm 1mm
	D1			Sign: CSign: /Sign: Csign:
	D2			Sed Type: sand mud clay
	D3			Odor/Color: fishy sulfur oily fecal / brown gray black
	D4			Other Notes: PODS
	D5			Press Sieved: 2mm 1mm
	E1			Sign: CSign: /Sign: Csign:
	E2			Sed Type: sand mud clay
	E3			Odor/Color: fishy sulfur oily fecal / brown gray black
	E4			Other Notes: PODS
	E5			Press Sieved: 2mm 1mm
	F1			Sign: CSign: /Sign: Csign:
	F2			Sed Type: sand mud clay
	F3			Odor/Color: fishy sulfur oily fecal / brown gray black
	F4			Other Notes: PODS
	F5			Press Sieved: 2mm 1mm
	G1			Sign: CSign: /Sign: Csign:
	G2			Sed Type: sand mud clay
	G3			Odor/Color: fishy sulfur oily fecal / brown gray black
	G4			Other Notes: PODS
	G5			Press Sieved: 2mm 1mm
	H1			Sign: CSign: /Sign: Csign:
	H2			Sed Type: sand mud clay
	H3			Odor/Color: fishy sulfur oily fecal / brown gray black
	H4			Other Notes: PODS
	H5			Press Sieved: 2mm 1mm

Data Entry: _____ QA'd: _____

ENVIRONMENTAL TESTING CENTER STANDARD OPERATING PROCEDURE

PREPARING TEST CHAMBERS FOR *AMPELISCA* TOXICITY TESTS

1. OBJECTIVE

- 1.1 This document describes the methods used to prepare test chambers for testing.
- 1.2 Test chambers are randomized, labelled and soaked in sea water the day before sediments are added.
- 1.3 Two hours are required to complete these procedures.

2. SAFETY

- 2.1 No special safety precautions are required.

3. MATERIAL

- 3.1 Seawater.
- 3.2 Test chambers and lids.
- 3.3 Labeling tape.
- 3.4 Waterproof marker.
- 3.5 Data Sheet AMP-051 "10-Day Solid-Phase -- Randomization Sheet" (attached).

4. METHODS

- 4.1 Prepare Data Sheet AMP-051.
 - 4.1.1 Create a RAND file in Excel by copying file 5 rebrand as Rand X, where X represents the test number.
 - 4.1.2 Save the file to the appropriate subdirectory.
 - 4.1.3 Assign a randomly chosen replicate number to each test chamber by drawing a numbered plastic chip.
 - 4.1.4 Enter in the "Rep" column.
 - 4.1.5 Double check that numbers have not been omitted or duplicated by copying the rep column, pasting it to an empty range at the bottom of the page and sorting it into ascending order.
 - 4.1.6 Fill in the experiment number and project in the header.
 - 4.1.7 Cursor right to randomization page.
 - 4.1.8 Highlight whole page.
 - 4.1.9 Edit, copy, paste special, values.
 - 4.1.10 On the far right edge, under "circles", sort the column (these are the first two reps) into ascending order:
 - 4.1.11 Highlight column, data, sort, enter.
 - 4.1.12 Repeat on the page below with the third replicates, in the column called "stars".
 - 4.1.13 SAVE THE FILE.
 - 4.1.14 Print out pages 3 and 4. These are the "randomization sheets".

- 4.2 Label the jars.
- 4.2.1 Attach waterproof colored tape to each jar above the word "Ball".
- 4.2.2 Turn under a corner of each tape so that it may be removed easily later.
- 4.2.3 Arrange the jars in groups of five on a cart (if available) or on a table.
- 4.2.4 Label each group of five jars with a group of five numbers from the randomization sheet, using a waterproof pen.
- 4.2.5 Double check that the correct five numbers have been assigned to each group of five jars. Sign Data Sheet AMP-051 at "randomization by."
- 4.2.6 On the labels, circle the numbers that correspond to the first two replicates of each group. These two replicates will be used for physical data measurements.
- 4.2.7 If it seems likely that the fourth and fifth reps will be preserved for sorting at a later date, the third rep must also be distinguishable. Put a star on the label of these jars.
- 4.2.8 Check Data Sheet AMP-051 and sign to assure that the jars are correctly assigned and that no numbers are duplicated.
- 4.3 Fill test chambers with seawater and cover. Allow to soak overnight.

10 Day Solid Phase Test--Randomization Sheet

Project: _____
Species: _____

Experiment #: _____

Jar #	Sort	Client #/Descriptor	Carboy #	Sample Description / Sample QA
	A1	LIS Control		Sign: CSign: /Sign: Csign:
	A2	LIS Control		Sed Type: sand mud clay
	A3	Jar #: LIS Control		Odor/Color: fishy sulfur oily fecal / brown gray black
	A4		Other Notes: PODS	
	A5		Press Sieved: 2mm 1mm	
	B1			Sign: CSign: /Sign: Csign:
	B2			Sed Type: sand mud clay
	B3			Odor/Color: fishy sulfur oily fecal / brown gray black
	B4			Other Notes: PODS
	B5			Press Sieved: 2mm 1mm
	C1			Sign: CSign: /Sign: Csign:
	C2			Sed Type: sand mud clay
	C3			Odor/Color: fishy sulfur oily fecal / brown gray black
	C4			Other Notes: PODS
	C5			Press Sieved: 2mm 1mm
	D1			Sign: CSign: /Sign: Csign:
	D2			Sed Type: sand mud clay
	D3			Odor/Color: fishy sulfur oily fecal / brown gray black
	D4			Other Notes: PODS
	D5			Press Sieved: 2mm 1mm
	E1			Sign: CSign: /Sign: Csign:
	E2			Sed Type: sand mud clay
	E3			Odor/Color: fishy sulfur oily fecal / brown gray black
	E4			Other Notes: PODS
	E5			Press Sieved: 2mm 1mm
	F1			Sign: CSign: /Sign: Csign:
	F2			Sed Type: sand mud clay
	F3			Odor/Color: fishy sulfur oily fecal / brown gray black
	F4			Other Notes: PODS
	F5			Press Sieved: 2mm 1mm
	G1			Sign: CSign: /Sign: Csign:
	G2			Sed Type: sand mud clay
	G3			Odor/Color: fishy sulfur oily fecal / brown gray black
	G4			Other Notes: PODS
	G5			Press Sieved: 2mm 1mm
	H1			Sign: CSign: /Sign: Csign:
	H2			Sed Type: sand mud clay
	H3			Odor/Color: fishy sulfur oily fecal / brown gray black
	H4			Other Notes: PODS
	H5			Press Sieved: 2mm 1mm

Data Entry: _____ QA'd: _____

ENVIRONMENTAL TESTING CENTER STANDARD OPERATING PROCEDURE

ADDING SEDIMENTS TO *AMPELISCA* TEST CHAMBERS

1. OBJECTIVE

- 1.1 The purpose of this document is to describe the methods used to add sediment to chambers for solid-phase testing.
- 1.2 To assure oxidation of the sediment surface, sediments and seawater are added to test chambers the day before the animals are introduced.
- 1.3 Four hours are required to complete these procedures.

2. SAFETY

- 2.1 Sediment and water samples should always be regarded as hazardous. They may contain toxins and/or infectious agents. Sediment handling operations must always be conducted in, or in front of, the fume hood.
- 2.1 Protective clothing to be worn include: safety glasses, face shields, latex, silvershield, and nitrile gloves, waterproof blue lab coat or poly laminated coveralls and poly lam apron, and dielectric boots.

3. MATERIALS

- 3.1 Sediment sample(s).
- 3.2 Control sediment.
- 3.3 Turbulence reducer(s).
- 3.4 Plastic spoons and spatulas.
- 3.5 Sponge.
- 3.6 Squeeze bottle filled with seawater.
- 3.7 Data Sheet AMP-061 "10-Day Solid-Phase -- Randomization Sheet" (attached).
- 3.8 Modified funnels (cut off at neck).
- 3.9 Electric drill with teflon coated paddle.
- 3.10 Data Sheet AMP-062 "Waste Log for Barrel Number X" (attached).

4. METHODS

- 4.1 Homogenize previously press sieved sediment sample using an electric drill and a Teflon coated paddle. (Not all samples will have been previously press sieved (see ETC SOP).
- 4.2 For each sediment sample check Data Sheet AMP-061 and select the appropriately numbered jars for that sample. Record the necessary information on the Data Sheet (ie., sample number, description, presence of amphipods).
- 4.3 Sign the Data Sheet matching your choice of sample to a group of five jars.
- 4.4 A second person **must** check the assignment of a sample to set of jars. They **must** also sign the Data Sheet.
- 4.5 Pour or scoop ca. 200 ml of homogenized sediment through a modified funnel into each test chamber using the metric markings already on the quart jars for measuring.

- 4.6 Gently tap test chamber or smooth sediment surface with a spatula to eliminate air pockets and surface irregularities.
- 4.7 Rinse all mud from sides of test chamber using a squeeze bottle filled with seawater at 30 ppt.
- 4.8 Use a turbulence reducer to slowly add seawater to test chamber to the 800 ml mark and place cover on chamber.
- 4.9 Transfer test jars to waterbath table.
- 4.10 Place test chambers in table in numerical order, in groups of five (to make air tubes easier to connect).
- 4.11 Put pipettes in test chambers so that the tip of the pipette is approximately half way down the water column (between the 400 and 600 ml mark).
- 4.12 Attach air lines to pipettes and turn on air pump.
- 4.13 Check all test chambers to make sure air is bubbling through pipettes.
- 4.14 Adjust 'gang' valves for gentle aeration, if necessary.
- 4.15 Add tap water to the water table, turn on the circulating pump and set the temperature control to 20°C. as soon as constant flow can be assured.
- 4.16 Rinse muddy utensils in the settling bucket.
- 4.17 Allow mud to settle overnight then decant the water down the drain.
- 4.18 Scoop the sediment out with a designated waste scoop and add to the appropriate 55 gallon waste drum. Add the necessary information to Data Sheet AMP-062.

10 Day Solid Phase Test--Randomization Sheet

Project: _____
Species: _____

Experiment #: _____

Jar #	Sort	Client #/Descriptor	Carbov #	Sample Description / Sample QA
	A1	LIS Control		Sign: CSign: /Sign: Csign:
	A2	LIS Control		Sed Type: sand mud clay
	A3	Jar #: LIS Control		Odor/Color: fishy sulfur oily fecal / brown gray black
	A4			Other Notes: PODS
	A5		LIS Control	Press Sieved: 2mm 1mm
	B1			Sign: CSign: /Sign: Csign:
	B2			Sed Type: sand mud clay
	B3			Odor/Color: fishy sulfur oily fecal / brown gray black
	B4			Other Notes: PODS
	B5			Press Sieved: 2mm 1mm
	C1			Sign: CSign: /Sign: Csign:
	C2			Sed Type: sand mud clay
	C3			Odor/Color: fishy sulfur oily fecal / brown gray black
	C4			Other Notes: PODS
	C5			Press Sieved: 2mm 1mm
	D1			Sign: CSign: /Sign: Csign:
	D2			Sed Type: sand mud clay
	D3			Odor/Color: fishy sulfur oily fecal / brown gray black
	D4			Other Notes: PODS
	D5			Press Sieved: 2mm 1mm
	E1			Sign: CSign: /Sign: Csign:
	E2			Sed Type: sand mud clay
	E3			Odor/Color: fishy sulfur oily fecal / brown gray black
	E4			Other Notes: PODS
	E5			Press Sieved: 2mm 1mm
	F1			Sign: CSign: /Sign: Csign:
	F2			Sed Type: sand mud clay
	F3			Odor/Color: fishy sulfur oily fecal / brown gray black
	F4			Other Notes: PODS
	F5			Press Sieved: 2mm 1mm
	G1			Sign: CSign: /Sign: Csign:
	G2			Sed Type: sand mud clay
	G3			Odor/Color: fishy sulfur oily fecal / brown gray black
	G4			Other Notes: PODS
	G5			Press Sieved: 2mm 1mm
	H1			Sign: CSign: /Sign: Csign:
	H2			Sed Type: sand mud clay
	H3			Odor/Color: fishy sulfur oily fecal / brown gray black
	H4			Other Notes: PODS
	H5			Press Sieved: 2mm 1mm

Data Entry: _____ QA'd: _____

**ENVIRONMENTAL TESTING CENTER
STANDARD OPERATING PROCEDURE**

SIEVING *AMPELISCA* FROM HOLDING JARS

1. OBJECTIVE

- 1.1 This document describes the methods used to sieve animals from holding jars used in the laboratory during acclimation.
- 1.2 Animals are sieved from holding jars for use in the 10-day solid-phase test.
- 1.3 Two hours are required to complete these procedures.

2. SAFETY

- 2.1 Dielectric boots must be worn if electric air pumps are used to aerate during this activity.

3. MATERIALS

- 3.1 One clean 12" 0.5 mm sieve per person.
- 3.2 One aquarium fish net per person.
- 3.3 One large glass dish per person.
- 3.4 One transfer container (plastic dish) per holding jar.
- 3.5 One specimen cups per holding jar.
- 3.6 Clean 5 gal buckets with holes in bottom.
- 3.7 Squeeze bottles filled with seawater (30 ppt salinity).
- 3.8 Large round settling bin.

4. METHODS

- 4.1 Run seawater into bin to the overflow level.
- 4.2 Fill large glass dishes half full with seawater.
- 4.3 Carefully pour off a small amount of water before bringing holding jar to bin.
- 4.4 Over the large bin, pour contents of holding jar into 0.5 mm sieve and sieve as usual (see ETC SOP). NOTE: Try to spray all tubes individually, so that very few animals remain in tubes.
- 4.5 Repeat process until no amphipods come out.
- 4.6 Rinse down tubes and place into specimen cup. DO NOT CAP.
- 4.7 Pour pods from dish into transfer container. DO NOT CAP.
- 4.8 Rinse net into sieve to ensure all animals have been retrieved.
- 4.9 Save tubes - transfer to specimen cups with same numbers as pods.
- 4.10 Repeat for each holding jar and be sure that the animals from each are kept in separate transfer containers (to keep track of any mortality).
- 4.11 Alternative method: Place bucket over floor grid. Place sieve on top. Spray tubes as above.
- 4.12 Immerse sieve in large bin to retrieve pods.
- 4.13 Rinse all equipment with deionized water. This includes holding jars and buckets.
- 4.14 Return all equipment to appropriate storage area.

- 4.15 Cap all transfer containers and specimen cups for transport to ETC for use.
- 4.16 At ETC pour contents of jars into separate, labelled dishes and aerate.

**ENVIRONMENTAL TESTING CENTER
STANDARD OPERATING PROCEDURE**

COUNTING *AMPELISCA* INTO TEST CHAMBERS

1. OBJECTIVE

- 1.1 The purpose of this document is to describe the methods used to count organisms for placement into exposure chambers.
- 1.2 Under normal testing procedures, 20 animals are placed into each test chamber. Reference test chambers are assigned 10 animals. These amphipods are randomly distributed to all specimen cups before being transferred to test chambers.
- 1.3 Two hours are required to complete this procedure.

2. SAFETY

- 2.1 No special safety concerns are required.

3. MATERIALS

- 3.1 *Ampelisca* from holding jars (see ETC SOP).
- 3.2 Clean wide bore pipettes with bulbs.
- 3.3 One specimen cup per test chamber and reference toxicant replicate.
- 3.4 One additional cup for a representative sample of animals to be preserved.
- 3.5 Clean seawater (30 ppt, 20°C).
- 3.6 Specimen cup with screen.
- 3.7 Squeeze bottle with seawater.
- 3.8 One glass dish per holding jar.
- 3.9 Data Sheet AMP-081 "Field Collection and Laboratory Holding" (attached).
- 3.10 Data Sheet AMP-082 "10-Day Solid-Phase Test -- Daily Data Sheet" (attached).

4. METHODS

- 4.1 Count out the number of specimen cups needed; one for each test chamber, one for each reference toxicant replicate, one for preserving a sample of animals, and a few extra for dead and gravid animals.
- 4.2 Fill each cup approximately half full of seawater.
- 4.3 Determine initial amphipod mortality in holding jars, for each jar used.
- 4.4 Remove all suspected dead amphipods. Determine condition under a stereomicroscope.
- 4.5 Record only the number of dead on Data Sheet AMP-081. NOTE: if mortality is greater than 5%, animals from that jar should not be used for testing.
- 4.6 Add animals to specimen cups.
 - 4.6.1 Twenty animals are added into each specimen cup in a random fashion, with each holding jar represented in each cup.

- 4.6.2 Determine the number of animals that can be used from each holding jar by dividing 20 by the number of holding jars. NOTE: the amphipods from each holding jar are now contained in a glass dish; one per holding jar (see ETC SOP).
- 4.6.3 Using a pipette select healthy looking, non-gravid amphipods two or three at a time, and place into specimen cups.
- 4.6.4 Switch glass dishes and add 2-3 more amphipods to each cup.
- 4.6.5 Continue switching dishes until each cup contains 10 amphipods.
- 4.6.6 Separate the number of cups needed for the reference test.
- 4.6.7 Continue to switch dishes until all remaining cups contain 20 animals. Note: if there are not enough animals the test may be performed with as few as 15 animals if the client agrees.
- 4.6.8 Examine every cup before proceeding.
- 4.6.9 Replace gravid or weak looking animals.
- 4.7 Add animals to test chambers.
- 4.7.1 Gently pour contents of specimen cup into a screened bottom transfer cup.
- 4.7.2 Verify amphipod count and check again for gravid females and remove, add additional amphipods from glass dishes if needed.
- 4.7.3 After count has been verified pour the amphipods from the screened bottom transfer cup into the test chamber using a squeeze bottle filled with 30 ppt , 20°C seawater.
- 4.7.4 Squirt sides of transfer cup and assure that all amphipods were transferred to test chamber.
- 4.7.5 Using the squeeze bottle again, squirt down the sides of the test chamber making sure all amphipods are in the water column.
- 4.7.6 Gently prod any floating amphipods with pipette so that the amphipods will swim down and bury into the sediment.
- 4.7.7 Check test chamber carefully for any amphipods stuck on sides of chamber or floating on surface.
- 4.7.8 Check all chambers after one hour for any "floaters" or any amphipods that have not buried into sediment and replace these.
- 4.7.9 Resume aeration.
- 4.7.10 Record any replacements made and time that aeration was resumed on Data Sheet AMP-082.

10 Day Solid Phase Test--Daily Data Sheet

Project: _____

Experiment #: _____

Day: _____ Date: _____

Organism: _____

Time/Initials: _____

Jar #	Observations*				Cum # Dead	Jar #	Observations*				Cum # Dead	Jar #	Observations*				Cum # Dead
	E	M	NMT	D			E	M	NMT	D			E	M	NMT	D	
1						31						61					
2						32						62					
3						33						63					
4						34						64					
5						35						65					
6						36						66					
7						37						67					
8						38						68					
9						39						69					
10						40						70					
11						41						71					
12						42						72					
13						43						73					
14						44						74					
15						45						75					
16						46						76					
17						47						77					
18						48						78					
19						49						79					
20						50						80					
21						51						81					
22						52						82					
23						53						83					
24						54						84					
25						55						85					
26						56											
27						57											
28						58											
29						59											
30						60											

Animals/rep: _____

Temp: _____ °C

Thermometer #: _____

Previous day's Cumulative number dead, QA'd by: _____

*KEY: E=emerged, M=molt, NMT=neuromuscular twitch, D=dead
Comments:

**ENVIRONMENTAL TESTING CENTER
STANDARD OPERATING PROCEDURE**

DAILY OBSERVATIONS OF AMPELISCA TOXICITY TEST

1. OBJECTIVE

- 1.1 This document describes the techniques used to perform daily observations of test chambers during the 10-day solid-phase test.
- 1.2 Daily observations are made on each test container to check for emerged or dead amphipods and the presence of any molts. Salinity, pH and dissolved oxygen are recorded twice during the test, once near the beginning (preferably on day one) and again near the end of the test (preferably on day nine).
- 1.3 One to two hours are required to complete these procedures.

2. SAFETY

- 2.1 Sediment and water samples should always be regarded as hazardous. They may contain toxins and/or infectious agents.
- 2.2 Aeration should be interrupted to sequential portions of the test table to reduce personnel exposure to aerosols.
- 2.3 At a minimum, safety glasses, white tyvek lab coats and latex gloves must be worn to check tests. If the samples are known to come from contaminated areas, waterproof blue lab coats must be worn.
- 2.4 When sewage samples are being tested, surgical masks should be worn for all operations.

3. MATERIALS

- 3.1 Data Sheet AMP-091 "10-Day Solid-Phase Test -- Daily Data Sheet" (attached).
- 3.2 Data Sheet AMP-092 "10-Day Solid-Phase Test -- Breakdown Sheet" (attached).

4. METHODS

- 4.1 Check temperature recorder and note any irregularities. Read regular thermometer and record temperature.
- 4.2 Interrupt aeration to first quarter of table by bypassing the first 4-5 gang valves.
- 4.3 Check test jars for emerged amphipods and molts.
- 4.4 Remove aeration pipette and lid from test chamber.
- 4.5 Rinse inside edge of chamber with seawater from squeeze bottle.
- 4.6 Look into chamber for emerged amphipods or molts and remove with a clean pipette.
- 4.7 Place amphipods/molts into petri dish and examine under dissecting microscope.
- 4.8 Emerged amphipods should be classified as one of the following: NOTE: *Dead*--usually exhibit the following: not curled up; body is soft; gut is empty; may be desintegrating; when gently touched with probe, the legs and antennae do not move; and there is no neuromuscular twitch; *Neuromuscular twitch (NMT)*--appears dead, but when gently touched with probe near the legs or midsection, one or two legs may kick spasmodically; *Emerged*--any live amphipod not burrowed in the sediment, i.e. floating, swimming, or

- lying on the surface of sediment; *Molt*--usually exhibits the following: transparent; no eyes; no gut; and appears hollow.
- 4.9 Return emerged and NMT animals to test chambers; dispose of molts and dead animals. Record on Data Sheet AMP-091.
 - 4.10 Replace lid and pipette on chamber.
 - 4.11 Between test chambers, rinse sampling pipette inside and out with seawater.
 - 4.12 When the first quadrant has been checked, resume aeration to the jars; interrupt aeration to the next quadrant.
 - 4.13 When all chambers have been checked, rinse probes, pipettes, etc. with deionized water and let air dry on a paper towel.
 - 4.14 Wipe down microscope and turn off light.
 - 4.15 Rinse dead pods down sink.
 - 4.16 Walk back along both sides of the table and assure that all lids and pipets have been replaced, and that all pipet are bubbling.
 - 4.17 Twice during the test, check dissolved oxygen concentration, pH and salinity in all pre-selected jars (see ETC SOP).
 - 4.18 End test on day 10.
 - 4.18.1 Check test as usual except emerged and NMT animals are placed into corresponding vials (instead of being returned to jars) and indicated on Data Sheet AMP-092.
 - 4.19 Rinse tools used to check test and place in dirty dish bin.

10 Day Solid Phase Test--Daily Data Sheet

Project: _____

Experiment #: _____

Day: _____ Date: _____

Organism: _____

Time/Initials: _____

Jar #	Observations*				Cum # Dead	Jar #	Observations*				Cum # Dead	Jar #	Observations*				Cum # Dead
	E	M	NMT	D			E	M	NMT	D			E	M	NMT	D	
1						31						61					
2						32						62					
3						33						63					
4						34						64					
5						35						65					
6						36						66					
7						37						67					
8						38						68					
9						39						69					
10						40						70					
11						41						71					
12						42						72					
13						43						73					
14						44						74					
15						45						75					
16						46						76					
17						47						77					
18						48						78					
19						49						79					
20						50						80					
21						51						81					
22						52						82					
23						53						83					
24						54						84					
25						55						85					
26						56											
27						57											
28						58											
29						59											
30						60											

Animals/rep: _____

Temp: _____ °C

Thermometer #: _____

Previous day's Cumulative number dead, QA'd by: _____

*KEY: E = emerged, M = molt, NMT = neuromuscular twitch, D = dead

Comments:

10 Day Solid Phase Test--Breakdown Data Sheet

Project: _____
 Species: _____

Experiment #: _____
 Date: _____

First Pick							QA - RePick				Final Count					
Initials	Time	Jar #	Dead During Test	Day 10 Emerged	# Live	# Dead	Recount			QA - RePick		Final Count				
							Initials	# Live *	# Animals Missing **	Initials	# Live	Initials	# Live	# Tubes Day 0	24 Hr. QA	Final # Live ***
		1														
		2														
		3														
		4														
		5														
		6														
		7														
		8														
		9														
		10														
		11														
		12														
		13														
		14														
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		18														
		19														
		20														
		21														
		22														
		23														
		24														
		25														
		26														
		27														
		28														
		29														
		30														

Comments: * # Live = the # live emerged on day 10 + the # live found by the picker
 ** If > 10% of the animals are missing (ie. > 2 of 20), the sample must be QA'd
 *** Final # Live = the # live from the 'first pick' recount + the # live from the QA recount

**ENVIRONMENTAL TESTING CENTER
STANDARD OPERATING PROCEDURE**

SIEVING *AMPELISCA* FOR TEST BREAKDOWN

1. OBJECTIVE

- 1.1 The purpose of this document is to describe the methods used to sieve amphipods from test sediments at the end of a test.
- 1.2 Sediment in each exposure chamber must be sieved and the contents of the sieve must be picked through in order to count surviving amphipods. If time does not permit immediate picking of all jars, a minimum of three replicates per sample must be picked live. Two may be preserved with formalin for later analysis.
- 1.3 Eight hours are required to complete these procedures.

2. SAFETY

- 2.1 Sediment and water samples should always be regarded as hazardous. They may contain toxins and/or infectious agents.
- 2.2 Sieving must be conducted in, or in front of, the hood in the preroom while wearing full protective gear (safety glasses and face shield, latex, silvershield, and nitrile gloves, waterproof blue labcoat or poly laminated coveralls and poly lam apron, and dielectric boots).
- 2.3 Formalin must be prepared and used in the hood.

3. MATERIALS

- 3.1 0.5 mm designated DIRTY sieve.
- 3.1 Plastic bin/basin.
- 3.2 Bucket with drainage hole.
- 3.3 Large dish.
- 3.4 Seawater squeeze bottle.
- 3.5 Pipette.
- 3.6 Forceps.
- 3.7 One medium size carolina dish (labelled the same as corresponding test chamber).
- 3.8 Graduated jars, with caps, numbered for the fourth and fifth rep of each sample - optional - used only if these reps are to be preserved.
- 3.9 Data Sheet AMP-101 "Waste Log" (attached).

4. METHODS

- 4.1 Select a test chamber and correspondingly numbered medium dish. NOTE: if two persons are sieving, one should take the even-numbered jars and one the odds.
- 4.2 Sieve out reps 1-3 for each sample first (labels have circles or stars).
- 4.3 Empty test chamber into sieve over the bucket.
- 4.4 Rinse jar thoroughly into sieve.

- 4.5 Rinse the sediment through sieve using a moderate force tap water spray, then rinse the remaining material in sieve with seawater squeeze bottle.
- 4.6 Flush out all tapwater and replace with seawater.
- 4.7 Place the labeled medium dish inside the large dish.
- 4.8 Use the seawater squeeze bottle to rinse the material from the sieve into the medium dish.
- 4.9 Check the large dish for any spillage, and pipette or pour into medium dish.
- 4.10 Gently submerge the sieve almost to the rim to make sure no pods or tubes remain on the sieve.
- 4.11 Use a pipette to transfer amphipods to the dish, use forceps for the tubes.
- 4.12 Slap the sieve forcefully against the surface of the water several times to dislodge any pods clinging to the screen.
- 4.13 Sink to the rim and examine the water surface for pods.
- 4.14 Repeat this process at least twice more, until all amphipods have been removed.
- 4.15 Invert the sieve over the bucket and backflush it forcefully with a stream of tapwater so that any overlooked pods will not be included in the next sample.
- 4.16 If it is necessary to preserve samples, rinse them into numbered graduated jars (special jars reserved for this use - do not use canning jars) with a minimum of seawater.
- 4.17 Note the total volume.
- 4.18 Add an equal volume of 10% formalin containing rose bengal stain and borax buffer
- 4.19 Clean up.
 - 4.19.1 Soak canning jars (exposure chambers) and other glassware and plasticware in dish bin full of tap water
 - 4.19.2 Rinse sieve and metal tools in deionized water.
 - 4.19.3 Hose down hood, wipe around sinks, sponge all sediment from water faucets, wash battles, etc.
 - 4.19.4 Allow sediment in bucket to settle overnight then decant water down the drain and transfer mud to 55 gallon waste drum with designated waste scoop.
 - 4.19.5 Fill in information on Data Sheet AMP-101.

**ENVIRONMENTAL TESTING CENTER
STANDARD OPERATING PROCEDURE**

PICKING *AMPELISCA* AT END-OF-TEST

1. OBJECTIVE

- 1.1 The purpose of this document is to describe the methods used to recover amphipods at the end of the 10-day solid-phase test.
- 1.2 At test termination, the material remaining on the 0.5 mm screen as each test chamber is sieved must be sorted under a stereomicroscope in order to recover the amphipods and assess their condition.
- 1.3 Sixty hours are required to complete this procedure.

2. SAFETY

- 2.1 Sediment and water samples should always be regarded as hazardous. They may contain toxins and/or infectious agents. Latex gloves and white tyvek or blue waterproof lab coats must be worn at all times. (Blue waterproof coats are preferable, and are required if the samples are known to come from a hazardous area.) Lab coats must be open in the back.
- 2.2 Alcohols and formalin will be used to preserve vials of animals. Wear gloves and work in the fume hood with either of these toxic, flammable materials. Flag the tray of vials with the appropriate printed hazard label.

3. MATERIALS

- 3.1 Dissecting microscope.
- 3.2 Wide bore pipette.
- 3.3 Dissecting utensils.
- 3.4 Forceps.
- 3.5 Data Sheet AMP-111 "10-Day Solid-Phase Test -- Breakdown Data Sheet" (attached).

4. METHODS

- 4.1 Select a sieved sample of sediment. Reps 1-3 of each sample should be sieved and picked before reps 4 and 5.
- 4.2 Look for amphipods.
 - 4.2.1 Look into dish for any floating amphipods.
 - 4.2.2 Agitate sediment and water to get any loose amphipods up to surface.
 - 4.2.3 Pour out excess water into another dish, making sure no amphipods escape.
- 4.3 Look for tubes.
 - 4.3.1 Pick through sediment and tubes using a stereomicroscope, a small portion at a time, removing amphipods, putting them into a separate dish.
 - 4.3.2 Put this sediment into the dish containing excess water.

- 4.3.3 Continue picking through sediment until all of sediment is gone.
- 4.4 With the microscope, examine the amphipods picked from the sample to determine how many are alive and how many are dead.
- 4.5 Transfer live amphipods into the appropriate scintillation vial with a minimum amount of seawater using a pipette.
- 4.6 Dead amphipods are noted on Data Sheet AMP-111 and then discarded.
- 4.7 Carefully record the following information on Data Sheet AMP-111 before continuing to another sample.
 - 4.7.1 Picker's initials and time completed picking.
 - 4.7.2 Jar number which corresponds to dish number and vial number.
 - 4.7.3 Number of live and dead amphipods.
 - 4.7.4 Number of amphipods lost- number of amphipods that cannot be accounted for among those found dead during the test, emerged on Day10, found live, and found dead.
 - 4.7.5 Add the number in the above categories and subtract them from 20 (or the number of animals added at start if a number other than 20 was added).
- 4.8 Return sediment to original dish.
- 4.9 A second person must verify the count of live animals found in each sample.
- 4.10 If multiple persons are picking at the same time, it is preferable to pass the petri plates to another person and have her/him immediately verify the count, and initial the Data Sheet. If this is not possible, the live animals must be transferred to an appropriately numbered vial to be counted later.
- 4.11 Any sample with more than ten percent (> 2 amphipods) lost should be segregated to be repicked.
- 4.12 Re-pick replicates with more than 10% of amphipods unaccounted for.
 - 4.12.1 Choose sample that you did not pick originally.
 - 4.12.2 Look for amphipods as above.
 - 4.12.3 Enter the number live, number dead and initials on Data Sheet AMP-111.
- 4.13 Clean up.
 - 4.13.1 Make sure light of microscope is turned off.
 - 4.13.2 Put picking tools and dishes into dirty dish bin.
 - 4.13.3 Wipe down spilled water and sediment.
 - 4.13.4 Clean off microscope and light.
 - 4.13.5 Put away safety clothing.

10 Day Solid Phase Test--Breakdown Data Sheet

Project: _____
 Species: _____

Experiment #: _____
 Date: _____

First Pick							Recount			QA - RePick		Recount		Final Count		
Initials	Time	Jar #	Dead During Test	Day 10 Emerged	# Live	# Dead	Initials	# Live	# Animals Missing	Initials	# Live	Initials	# Live	# Tubes Day 0	24 Hr. QA	Final # Live
		1														
		2														
		3														
		4														
		5														
		6														
		7														
		8														
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		30														

Comments: * # Live = the # live emerged on day 10 + the # live found by the picker
 ** If >10% of the animals are missing (ie. > 2 of 20), the sample must be QA'd
 *** Final # Live = the # live from the 'first pick' recount + the # live from the QA recount

ENVIRONMENTAL TESTING CENTER STANDARD OPERATING PROCEDURE

PICKING PRESERVED SAMPLES

1. OBJECTIVE

- 1.1 The purpose of this document is to describe the methods used to pick amphipods from preserved samples.
- 1.2 Samples are preserved at the end of the 10-day solid-phase test when time is not available for live picking. These samples are preserved with borox-buffered 10% formalin with Rose-Bengal used as a live stain. Live amphipods should stain bright fuschia/pink.
- 1.3 Thirty minutes are required to pick through one sample.

2. SAFETY

- 2.1 Sediment and water samples should always be regarded as hazardous. They may contain toxins or infectious agents.
- 2.2 Formalin is toxic, flammable, and causes cancer. Preserved samples must be handled only in the hood or in fume guards. Wear a lab coat, latex gloves, and safety glasses and face shield when resieving samples.
- 2.3 The face shield and glasses may be removed at the microscope.
- 2.4 Formalin is so difficult to remove from glassware that we have an entirely separate set of sieving and picking tools to use with preserved samples. These tools are kept under the fume hood and must be returned to this spot after use. It is very important not to mix them with tools used for live work.

3. MATERIALS

- 3.1 Fume guard.
- 3.2 Dissecting microscope.
- 3.3 Wide bore pipette.
- 3.4 Dissecting utensils.
- 3.5 Forceps.
- 3.6 "Formalin only" 0.5 mm sieve.
- 3.7 Formalin waste bin.
- 3.8 Formalin disposal cannister.
- 3.9 Plastic dishes for holding sieved sample.
- 3.10 Data Sheet AMP-121 "10-Day Solid-Phase -- Breakdown Sheet" (attached).
- 3.11 Squeeze bottle.

4. METHODS

- 4.1 Select a preserved sediment sample jar.
- 4.2 Check Data Sheet AMP-021 to assure that it has not been picked already.
- 4.3 Sieve formalin sample out to remove excess formalin.

- 4.3.1 Turn hood on.
- 4.3.2 Sieve sample through the "formalin only" 0.5 mm sieve over blue formalin waste bin.
- 4.3.3 Rinse into the bin with several small tap water rinses to remove most of the formalin.
- 4.3.4 Rinse sieve thoroughly with tap water hose over sink, using gentle pressure, being careful to retain all material on sieve. NOTE: Do not splash any material from the sieve.
- 4.3.5 Use a squeeze bottle filled with seawater to spray the material on the sieve into plastic dish.
- 4.3.6 Rinse sieve again to be sure all material has been transferred from the sieve to the "picking" dish.
- 4.4 Bring dish containing the sieved sample to the picking station, where fume guard is located.
- 4.5 Turn on the fume guard.
- 4.6 Keep the sample and waste containers well under the canopy at all times.
- 4.7 Look for bright fuchsia/pink amphipods.
- 4.7.1 Look into dish for any floating amphipods.
- 4.7.2 Agitate sediment and water to get any loose amphipods up to surface.
- 4.7.3 Pour out excess water into another dish, making sure no amphipods escape.
- 4.8 Look for tubes.
- 4.9 Use a stereomicroscope, to pick through the sediment and tubes.
- 4.10 Examine a small portion at a time.
- 4.11 Remove amphipods into a separate dish.
- 4.12 Put the sediment into a second dish.
- 4.13 Continue picking through sample until all of sediment is gone.
- 4.14 Pipette all amphipods into the appropriate scintillation vial with a minimum amount of seawater.
- 4.15 Carefully record the following data on Data Sheet AMP-121 before continuing to another sample.
 - 4.15.1 Put date in left hand margin of page.
 - 4.15.2 Pickers initials and time picking was completed.
 - 4.15.3 Number of amphipods dead during test (cumulative dead).
 - 4.15.4 Number emerged (pods found live before sieving, now in vials).
 - 4.15.5 Number live - preserved pods found in good condition.
 - 4.15.6 Number dead - list only animals in poor looking condition that did not stain well.
 - 4.15.7 Number lost - number of amphipods that cannot be accounted for among those (i) found dead during the test (ii) emerged on Day 10 (iii) found live (iv) found dead.
 - 4.15.8 Add the number of animals in the above categories and subtract them from 20 (or the number of animals added at test start, if different from 20).
- 4.16 Any sample with more than ten percent (> 2 amphipods) unaccounted for should be marked in the QA, and will be repicked by someone else.

10 Day Solid Phase Test--Breakdown Data Sheet

Project: _____
 Species: _____

Experiment #: _____
 Date: _____

First Pick							Recount			QA - RePick		Recount			Final Count		
Initials	Time	Jar #	Dead During Test	Day 10 Emerged	# Live	# Dead	Initials	# Live	# Animals Missing **	Initials	# Live	Initials	# Live	# Tubes Day 0	24 Hr. QA	Final # Live ***	
		1															
		2															
		3															
		4															
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		29															
		30															

- Comments:
- * # Live = the # live emerged on day 10 + the # live found by the picker
 - ** If > 10% of the animals are missing (ie. > 2 of 20), the sample must be QA'd
 - *** Final # Live = the # live from the 'first pick' recount + the # live from the QA recount

APPENDIX C:

HEALTH AND SAFETY PLAN

FOR

**FIELD COLLECTION OF CHEMISTRY AND BIOLOGY SAMPLES IN
CONTAMINATED AREAS IN THE VICINITY OF THE NAVAL EDUCATION CENTER,
NEWPORT, RI**

Revised July 8, 1994

**Prepared by:
Gregory A. Tracey
Science Applications International Corporation
165 Dean Knauss Drive
Narragansett, RI 02882**

This Safety and Health Plan is prepared to comply with OSHA regulation 29 CFR 1910.120, "Hazardous Waste Site Operations and Emergency Response," as it applies to sample collection activities to be conducted by URI/SAIC personnel at a Superfund or other uncontrolled hazardous waste site.

Approved by:

James M. Quinn
URI Project Manager

8/17/94
Date

Reviewed by:

Gregory C. Gray
Environmental Compliance
& Health and Safety Manager

8/15/94
Date

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1.0 Introduction

This Health and Safety Plan (HSP) establishes procedures and general guidelines for worker and public safety to be used by University of Rhode Island (URI) and Science Applications International Corporation (SAIC) personnel during field operations for the studies associated with collection of chemistry samples, namely water, organisms, or the deployment of monitoring organisms, in an environment contaminated with polychlorinated biphenyls (PCBs) and other hazardous substances.

1.1 Scope and Applicability

This HSP covers only the field collection activities conducted in support of ecological monitoring related to the scope of Task Order # (), under Navy CLEAN Contract No. N62472-90-D-1298, Contract Task Order 173 to Hallibuton NUS. Laboratory safety and health requirements applicable to this project are described in other documents.

1.2 Site and Project Scope of Work Overview

Activities within this project will assess the presence, toxicity, bioavailability, and bioaccumulation of various chemicals by examining sediments, deployed clean animals or looking at *in situ* organisms. It may also sometimes be necessary to participate in sample collection at onshore sites to evaluate these processes. The field studies will be conducted in the Lower East Passage of Narragansett Bay, with specific attention to areas around Coddington Cove and McAllister Point. These latter locations are adjacent to upland areas which have been designated as Superfund Sites on the National Priority List. Sediments adjacent to these areas have been found to be contaminated with PCBs and other hazardous substances.

The scope of work will require that chemists and biologists collect organisms and sediments to support the ecological risk assessment. These collections will occur during scheduled cruises presently planned for August through October 1994. In addition, SCUBA diving methods may be employed for instrument deployment and sample collection.

1.3 Site Description

Sample collection will occur in approximately eight main areas, three of which are expected to be contaminated (Inner and Outer Coddington Cove, and McAllister Point). URI and SAIC efforts related to sediment and organism collections will be accomplished either from a small boat (19 foot Boston Whaler) or larger vessel (45' fishing trawler) as appropriate. Other sediment and organism collection may also

be done from the shore. On-shore sample collection at the NETC Sites is not done by URI/SAIC but rather through Haliburton NUS. It however may be necessary for URI/SAIC team members to observe onshore sampling for purposes of ensuring data comparability between onshore and offshore components of the project. In addition, URI/SAIC team may receive split samples for chemical analyses.

2.0 PROJECT ORGANIZATION AND RESPONSIBILITIES

2.1 Project Organization

Halliburton NUS Project Manager:	Mr. Steve Parker ()
University of Rhode Island Project Manager:	Dr. James Quinn (401) 792-6219
SAIC Project Officer:	Dr. Wayne Munns (401) 782-1900
Site Safety and Health Officer:	Dr. Greg Tracey (SAIC) (401) 782-1900
Client Site Safety and Health Officer:	Mr. Brad Wheeler (NETC) (401) 841-3735
Contractor on Site:	NONE
Government Agency Representative on Site:	Mr. Brad Wheeler (NETC) (401) 841-3735
Field Team Leaders:	Dr. John King (URI) (401) 792-6594
Boat Operations Leader:	Mr. Mark Gustafson (URI) (401) 792-6205

All field team members are required to follow the requirements of this plan. Each field team member must sign the "Acknowledgement of Understanding" indicating they have read and fully understand these requirements. Field team members include:

Dr. Jim Quinn

Dr. John King
Dr. Wayne Munns
Dr. Greg Tracey
Technical Support Personnel (TBA)

2.2 Responsibilities

The URI Program Manager and for subcontracted activities, the SAIC Project Manager, has overall responsibility for the safe performance of tasks designated in the Work Plan, and is responsible for providing the resources needed to implement this plan and for enforcing its requirements.

The Site Health and Safety Officer is responsible for notifying the Project Managers when there are unexpected hazardous conditions, deviations from this plan, or violations of SAIC EC&HS requirements; maintaining project safety and health records; ensuring that protective clothing and equipment are properly used and maintained; ensuring that personnel performing field work have completed the required training and have medical clearance; and conducting a post field activity debriefing to identify problems encountered and lessons learned. If the Site Safety and Health Officer determines that conditions in the field are unsafe, he has the authority to suspend field operations until the problem is corrected.

Each employee is responsible for:

- not accepting a task or responsibility without first understanding the risks and hazards associated with it;
- obtaining required training and providing a copy of training certificates to the Site Safety and Health Officer;
- obtaining required medical examination(s) and providing evidence of medical clearance to the Site Safety and Health Officer;
- knowing emergency response procedures;
- wearing and maintaining personal protective equipment as specified in this plan;
- reporting to the Site Safety and Health Officer any hazards not documented or inadequately controlled by this plan;
- immediately reporting any occupational illness or injury to the appropriate supervisor and/or to Personnel, including any potential exposure to hazardous substances for which protection was not

provided;

The URI Standard Operating Procedure "Safety Standards for URI/GSO: Small Boat Fleet (1989) (provided as an attachment to this Appendix) requires that where boats are used, a person shall remain on shore who knows the details of the trip, the persons involved, and the expected time of return, in case of an emergency. The individual designated this responsibility is:

James Latimer
(401) 792-6615

3.0 Task Description

3.1 Notifications Prior to Field Work

Prior to any field activities, Dr. Jim Quinn will notify NETC's Superfund Site Coordinator, Mr. Brad Wheeler, of collection procedures and dates. Personnel will also notify the Rhode Island Department of Environmental Management of the upcoming collection, location, and the URI permit number.

3.2 Field Activities

Sediment and Organism Collection: Sediment samples will be taken by box core. A team of scientists including Dr. Jim Quinn, Dr. John King, Dr. Greg Tracey and designated technical support personnel will subsample surface sediments and archive these samples for laboratory processing. Organism collections will be performed by sediment grabs or by SCUBA Diver as appropriate. All diving activities will conform to established Standard Operating Procedures.

Mussel Deployments: A team of one biologist (Dr. Greg Tracey) and one boat operator (TBA) will deploy and/or retrieve a polyethylene mesh bags of mussels that are suspended one meter above the sediment in the water column. Four bags are deployed/retrieved from each site. There is no contact with the sediment as the weight on the bottom of the array never is pulled completely out of the water. Mussels may be deployed for up to 28 days depending upon the monitoring and research requirements. The station locations are outlined in the Work Plan.

Other collections: One chemist (Dr. John King) will collect sediment cores from various stations, following established Standard Operating Procedures. In addition, some sample collections may occur in areas easily reached from the shore. These collections are not at standard stations and may involve access to restricted areas. All personnel accessing these areas will follow the established notification procedures and will have completed the OSHA 40-hr Hazardous Waste Site

Workers Training. One of these personnel will also have completed the 8-hr Supervisors training if one is not present at the site for sampling.

In all cases, after work has been completed at the contaminated site, all personnel will remove protective clothing and wash hands and face with decon soap and fresh water. Contaminated equipment will be wiped clean with paper towels, then washed with Decon soap. Contaminated debris and rinsewater (*i.e.*, cleaning materials, coveralls, *etc.*) will be collected separately, labeled with its contents (*i.e.*, decon water containing PCBs level of PCBs and accumulative start date) and transported back to the laboratory.

The boat operator will determine whether meteorological conditions are unsafe (*e.g.*, forecast of fog, heavy rains, thunder storms or extremely high winds). If conditions are unsafe, the trip will be postponed for the day.

Personnel will transport sediment and biota samples to the SAIC and URI laboratories in sealed ziplock bags, glass jars, or teflon jars as appropriate and will be contained in a large coolers. Personnel will hold samples in designated refrigerators and freezers as appropriate until analysis is begun.

4.0. Hazard Analysis

4.1 Potential Chemical Exposures

Hazardous substances in the harbor and nearshore sediments have been identified and quantified at several locations (see section 7.5.2. Site Characterization). Hazardous substances identified in the sediments include PCBs, petroleum hydrocarbons (PHCs), PAHs and several metals.

The highest contaminant concentrations in the sediments are located in Coddington Cove and off of McAllister Point. While sediment concentrations are extremely patchy, total PAH concentrations have been found to be as high as 30,000 $\mu\text{g}/\text{kg}$ dry weight ($\mu\text{g}/\text{kg}$ is equivalent to parts per billion). PCBs have also been measured in the sediment at McAllister Point up to 219 $\mu\text{g}/\text{kg}$, while Butyltins were measured at 88 $\mu\text{g}/\text{kg}$ dry weight at the Old Fire Fighting Training area. Among the metals, highest concentrations for As, Cr, Ni and Pb were 240, 359, 110, and 12900 $\mu\text{g}/\text{g}$ dry weight respectively, and were found at the McAllister Point site. Airborne levels of these contaminants have not been quantified.

None of the metals are volatile, nor are they absorbed readily through skin contact. The PCBs, PAHs, and tributyltins are all chloracne producing chemicals and may be absorbed through the skin. Exposure limits are shown in Table 1.

4.2 Potential Physical Hazards

Potential safety hazards associated with operating of small boats include capsizing, and consequent hypothermia and possible drowning. URI has developed safety procedure for boat use and will also be adhered to for those personnel using the boat.

4.3 Task Hazard Analysis

Most activities planned in this project involve only those safety hazards normally associated with boat use. The one exception is the collection of organisms or samples from the shore. Exposure to PCBs and other hazardous materials, primarily through skin contact with sediment (if unprotected), could occur during these activities. There is also some potential that during the collection or retrieval of organisms water droplets with some organic contamination could splash onto nearby surfaces.

Inhalation does not appear to be a significant route of exposure, compared with skin contact, because the chemicals of concern will not evaporate readily, and no processes are anticipated that will generate high aerosol concentrations. Therefore, the primary means of hazard control should be through wearing protective clothing that will prevent skin contact with sediments, organisms and contaminated water.

5.0 Hazard Monitoring and Control

5.1 Employee Training

Field team personnel will have completed a 40-hour hazardous waste site training course and an annual 8-hour refresher course if more than 12 months have elapsed since completing the 40-hour course. The field supervisor will have

Table 1. Exposure Limits

Substance	Highest Reported Sediment Concentration ($\mu\text{g}/\text{kg}$)	OSHA PEL (8-hr) (mg/m^3)	ACGIH TLV (8-hr) (mg/m^3)	Skin Notation	
				OSHA	ACGIH
PCBs (total)					
42% chlorine	-	1.0	1.0	Yes	Yes
54% chlorine	219	0.5	0.5	Yes	Yes
Petroleum hydrocarbons	2090	-	-	-	-
Butyltins	88	-	-	-	-
PAHs (total)	30000	.02 ^a	0.2 ^a	No	No
Arsenic	240		0.05	No	No
Chromium	359	1.0	0.05 ^b	No	No
Copper	1140	1.0	1.0 ^c	No	No
Mercury	10.7				
Lead	880	0.005	0.15 ^d	No	No
Nickel	138	1.0	1.0 ^e	No	No
Zinc	1580	10.0 ^f	10.0 ^f	No	No

completed an 8-hour manager/supervisor training course as required by 29 CFR 1910.120 for supervision of hazardous waste operations.

Additionally, a briefing on small boat handling will be provided to field team personnel.

Field team personnel will read this HSP and acknowledge their understanding of its content and requirements.

5.2 Personal Protective Equipment and Clothing

Available chemical hazard information indicates that the primary health hazard is incidental contact with sediment and sea water during organism collection or mussel deployment or retrieval. The risk of inhalation exposure to chemicals at concentrations exceeding permissible exposure limits is low. The level of protection judged adequate for planned task activities is Level D. The required personal protective equipment includes the following:

- Tyvek coveralls
- Latex inner gloves
- Nitrile outer gloves
- Rubber boots (knee high)
- Face shield

The level of protection may be upgraded to level C at the discretion of the SSHO or upon request of any field team member, if it appears that conditions warrant respiratory protection (e.g., calm water, no wind, high temperature, full sun, and numerous slicks surrounding the boat). In this case, a half-face or full-face air purifying respirator equipped with organic vapor cartridges may be used. All personnel using respirators will have received respirator use training, an annual fit test and medical approval per URI/SAIC SOPs.

The use of a sun screen preparation to reduce the potentially harmful effects of ultraviolet solar radiation is recommended. A protection factor of 15 or greater should be used.

5.3 Medical Surveillance

Personnel participating in field activities associated with hazardous waste operations must have annual medical examinations. The content of these examinations and related recordkeeping requirements can be found in the in the URI personnel manual or SAIC's EC&HS Manual Procedure 12. Medical Surveillance.

5.4 Monitoring and Sampling Plan

No air monitoring is planned for this activity.

5.5 Site Control Measures

Control of access to the site is accomplished by controlling access to the boat. The site has many land accesses (roads, parking lots) and the Navy has not attempted to limit access from land. Personnel who are designated as field team personnel in this HSP must be the only personnel allowed to accompany the field team during sediment or organism collection efforts.

5.6 Decontamination Plan

When the boat returns to the boat launch, personnel will take off protective clothing and wash hands and face with decon soap and fresh water. Contaminated equipment will be wiped clean with paper towels, then washed with Decon soap. Cleaning materials, coveralls, gloves, etc. will be placed into trash bags and returned to the URI for proper disposal by the Health and Safety Designee. Containers will be labelled with their contents (*i.e.*, used PPE/Equipment, containing ppm levels of PCBs).

Mussel arrays are always left in place and therefore do not require decontamination. Mussels are removed from the mesh bags (or organisms removed from their substrate) using a gloved hand and placed in a ziplock bags. The boat will be washed with Decon soap on its return to the laboratory. The contaminated collection materials (mesh bags in particular) will be returned to the lab, and double bagged, labelled, and returned to URI for proper management. All decon water will be collected, containers properly labeled and turned over to the URI Safety Designee for proper management.

5.7 Confined Space Entry

Confined space entry is not required for proposed task activities.

5.8 Sanitation

The short duration of this task precludes establishing temporary showers at the site. Arrangements have been made with the NETC to use showers at the Base in an emergency. The phone number is:

Mr. Brad Wheeler (401) 841-3735

5.9 Hazardous Waste Management

Sea water, sediment, and local material associated with the organisms may contain high organic and metal concentrations. OSHA requirements for sampling at superfund sites requires that nothing be thrown over the side of the boat except excess materials actually being sampled (water, shellfish, sediments, etc.). Any refuse accidentally collected (tires, cans, plastics, etc.) must be held and returned to URI for disposal. Contaminated debris and rinsewater (i.e., cleaning materials, coveralls, etc.) will be collected separately in 5-gallon containers, each container labeled with its contents (i.e., decon water containing PCBs, level of PCBs, accumulation start date) and transported back to URI. Waste associated with this task will be turned over to the URI Safety Designee for proper management.

5.10 Environmental Protection

Collection of organisms from shore will be done only in areas already disturbed by current anthropogenic activity. Most of the sites where this safety plan would be applicable are already highly contaminated and any activity outlined in this plan would not negatively impact the area.

6.0 Emergency Response Plan

6.1 Anticipated Emergencies

Personal injury, capsizing the boat, man overboard, grounding of the boat, and motor failure, hypothermia (if overboard), heat related illness (in hot weather) are potential emergency situations during this task.

6.2 Nearest Emergency Medical Facility

A medical facility has not been designated. Location of the nearest facility will depend on location of the boat. In the event of an emergency, the emergency dispatcher will be relied on to identify the nearest medical facility.

6.3 Emergency Communications

The URI procedure for boat operations which specifies that radios are to be used for emergency communication will be adhered to.

6.4 Emergency Phone Numbers and Radio Call Signs

Emergency Telephone: 911
Radio:
URI Call Sign (TBD)

Marina frequency, call sign: (TBD)
US Coast Guard on channel 16.

6.5 Emergency Equipment

The following emergency equipment must be placed aboard the boat and verified prior to departure:

- Radio on the Coast Guard frequency
- First Aid Kit
- Drinking Water (2 gallons/person/day)
- Fire Extinguisher (5 pound 2A-10BC)

6.6 On-site Emergency Procedures:

Personal Injury or Illness:

Administer First Aid; call ambulance; if necessary transport to hospital.

Fire or Explosion:

Follow instructions on fire extinguisher. Abandon the boat if incipient fire cannot be controlled.

Hazardous Material Spill or Release:

Leave the work area in a direction upwind of the spill or release; contact Coast Guard or other response personnel as necessary.

Other Equipment Failure:

If the boat fails to operate properly, the project team leader and site safety officer shall be notified and they shall determine the effect of this failure on continuing operations on site. If the failure affects the safety of personnel or prevents the proper completion of the tasks described in the work plan, all operations will be secured and all personnel shall leave the area until the situation has been evaluated and appropriate actions have been taken.

6.7 Accident Investigation Report

In the case of any injury, illness, or other accident affecting a URI/SAIC employee during this task survey, an Accident Investigation Report will be completed by the affected employee and the Site Safety Officer. The report will be immediately forwarded to the appropriate Local EC&HS Officer. An

Accident Investigation Report form is included in the SAIC's EC&HS Manual, Procedure 4. Accident Reporting.

7.0 References

American Conference of Governmental Industrial Hygienists, 1992-1993 Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices, Cincinnati, OH, 1992.

Department of Labor, Code of Federal Regulations, 29 CFR 1910.120, "Hazardous Waste Operations and Emergency Response," U.S. Government Printing Office, Washington, D.C.

Department of Labor, Code of Federal Regulations, 29 CFR 1910, Subpart Z, "Toxic and Hazardous Substances," U.S. Government Printing Office, Washington, D.C.

Phillips, C.F. and James, R.K., "Gasoline Vapor Exposure During Bulk Handling Operations," American Industrial Hygiene Association J. (39) pp 118-128, February, 1978.

Science Applications International Corporation, "Environmental Compliance & Health and Safety Program - Tailored Program", May, 1992.

Williams, P.L., & Burson, J.L. (1985). Industrial Toxicology, pp 156, New York: Van Nostrand Reinhold.

ACKNOWLEDGEMENT OF UNDERSTANDING

We, the undersigned, have read this Site Health and Safety Plan and will institute the provisions and abide by the regulations contained herein for the duration of this program.

	<u>Name (print)</u>	<u>Signature</u>	<u>Employee No.</u>	<u>Date</u>
1.	_____	_____	_____	_____
2.	_____	_____	_____	_____
3.	_____	_____	_____	_____
4.	_____	_____	_____	_____
5.	_____	_____	_____	_____
6.	_____	_____	_____	_____
7.	_____	_____	_____	_____