Chesapeake Bay Water Quality Monitoring Program

Long-Term Benthic Monitoring and Assessment Component Quality Assurance Project Plan

2021-2022

Prepared for

Maryland Department of Natural Resources
Tidewater Ecosystem Assessment
Annapolis, Maryland

Prepared by

Versar, Inc.
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May 2021

CHESAPEAKE BAY
WATER QUALITY MONITORING PROGRAM
LONG-TERM BENTHIC MONITORING
AND ASSESSMENT COMPONENT
QUALITY ASSURANCE PROJECT PLAN
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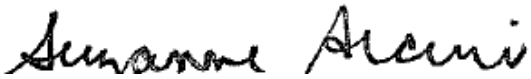

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FOREWORD

This document, *Chesapeake Bay Water Quality Monitoring Program: Long-Term Benthic Monitoring and Assessment Component, Quality Assurance Project Plan 2021-2022,* was prepared by Versar, Inc., at the request of Tom Parham of the Maryland Department of Natural Resources under Biomonitoring Contract # KOOB6400098 between Versar, Inc., and Maryland DNR. The document describes Standard Operating Procedures for the Maryland Department of Natural Resources Program which assesses the status of Chesapeake Bay benthic communities and evaluates their responses to changes in water and sediment quality.

TABLE OF CONTENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1.0 INTRODUCTION

1.1 BACKGROUND

Monitoring is a necessary part of environmental management as it provides the means for assessing the effectiveness of previous management actions and the information necessary to focus future actions (NRC 1990). Towards these ends, the State of Maryland has maintained a water quality and biological monitoring program for Chesapeake Bay since 1984. The goals of the program are to:

- quantify the types and extent of water quality problems (i.e., characterize the "state-of-the-bay");
- determine the response of key water quality measures to pollution abatement and resource management actions;
- identify processes and mechanisms controlling the bay's water quality;
- define linkages between water quality and living resources;
- contribute information to the Chesapeake Bay Health and Restoration reports; and
- contribute information to the Water Quality Characterization Report (305b report) and the List of Impaired Waters (303d list).

The program includes elements to measure water quality, sediment quality, phytoplankton, and benthic invertebrates. The monitoring program includes assessments of biota because the condition of biological indicators integrates temporally variable environmental conditions and the effects of multiple types of environmental stress. In addition, most environmental regulations and contaminant control measures are designed to protect biological resources; therefore, information about the condition of biological resources provides a direct measure of the effectiveness of management actions.

The Maryland program uses benthic macroinvertebrates as biological indicators because they are reliable and sensitive indicators of habitat quality in aquatic environments. Most benthic organisms have limited mobility and cannot avoid changes in environmental conditions (Gray 1979). Benthos live in bottom sediments, where exposure to contaminants and oxygen stress are most frequent. Benthic assemblages include diverse taxa representing a variety of sizes, modes of reproduction, feeding guilds, life history characteristics, and physiological tolerances to environmental conditions; therefore, they respond to and integrate natural and anthropogenic changes in environmental conditions in a variety of ways (Pearson and Rosenberg 1978; Warwick 1986; Wilson and Jeffrey 1994; Dauer 1993).

Benthic organisms are also important secondary producers, providing key linkages between primary producers and higher trophic levels (Virnstein 1977; Holland et al. 1980, 1989; Baird and Ulanowicz 1989; Diaz and Schaffner 1990). Benthic invertebrates are among the most important components of estuarine ecosystems and may represent the largest standing stock of organic carbon in estuaries (Frithsen 1989). Many benthic organisms, such as oysters and clams, are economically important. Others, such as polychaete worms and small crustaceans, contribute significantly to the diets of economically important bottom- feeding juvenile and adult fishes, such as spot and croaker (Homer et al. 1980; Homer and Boynton 1978).

The Chesapeake Bay Program's decision to adopt Benthic Community Restoration Goals (Ranasinghe et al. 1994, updated by Weisberg et al. 1997; Alden et al. 2002) enhanced use of benthic macroinvertebrates as a monitoring tool. Based largely on data collected as part of Maryland's monitoring effort, these goals describe the characteristics of benthic assemblages expected at sites exposed to little environmental stress. The Restoration Goals provide a quantitative benchmark against which to measure the health of sampled assemblages and ultimately the Chesapeake Bay. Submerged aquatic vegetation (Dennison et al. 1993) and benthic macroinvertebrates are the only biological communities for which such quantitative goals have been established to date in Chesapeake Bay.

1.2 OBJECTIVES OF THIS DOCUMENT

This document describes Standard Operating Procedures for all aspects of the Long-Term Benthic Monitoring and Assessment component (LTB) of the Maryland Department of Natural Resources Chesapeake Bay Water Quality Monitoring Program. The procedures ensure that data produced address the questions which the program is designed to answer. They include data quality objectives to ensure that all aspects of the program, from positioning for sample collection to the taxonomic level of identification of biota in samples, meet standards of accuracy and precision required to answer these questions.

1.3 ORGANIZATION OF THIS DOCUMENT

This document is organized into 8 Chapters. Chapter 2.0 states the Benthic Program objectives. Chapter 3.0 describes program management, organization, and the areas of responsibility of program personnel. Chapter 4.0 describes the field program including site selection, field measurements, and instrument calibration. Chapter 5.0 provides an overview of laboratory procedures and data quality objectives; specific steps for each procedure are described in the Versar, Inc. Laboratory Standard Operating Procedures Manual (Attachment 1). Chapter 6.0 describes data quality assurance procedures; it emphasizes data management and simplistic value checks because data quality controls are built into many aspects of the program. Chapter 7.0 provides an overview of standard statistical and graphical analysis techniques as well as standard products included in reports. Chapter 8.0 is a list of the literature cited.

2.0 OBJECTIVES

2.1 PROGRAM OBJECTIVES

The Maryland Long-Term Benthic Monitoring and Assessment Component has two primary objectives:

1) To assess status and trends in benthic community condition at 27 fixed sites located in the Maryland Bay. Sites were selected in multiple habitats distributed in sub-estuaries throughout the Maryland Bay in areas where the Bay was expected to respond to regulatory and management activities. Many of these sites have been sampled continuously since 1984. Sampling activities at these sites are described in Section 4.1.1.

2) To assess the area of the Bay supporting healthy benthic communities and identify benthic areas most in need of restoration. This is accomplished by assessing samples from probability sites selected using the stratified random sampling design described in Section 4.1.2.

From time to time, additional objectives are defined and addressed by special sampling programs at special sites, as described in Section 4.1.3.

The program is designed to answer the following questions:

1) What is the status and trend in benthic community condition at each fixed site? Is benthic condition changing, and if so, is it improving or degrading?

2) What is the area with good benthic community condition and the area with degraded benthic community condition in the Maryland portion of the Bay and in each of six subdivisions (strata)?

These questions are answered by applying the benthic index of biotic integrity and the Benthic Community Restoration Goals, upon which the index is based, to the data collected at the fixed and the probability sites.

2.2 DATA QUALITY OBJECTIVES

The performance and acceptance criteria that clarify objectives, define appropriate types of data, and specify tolerable levels of error are stated in Sections 4.0 (Field Program), 5.0 (Laboratory Processing), 6.0 (Data Management), and 7.0 (Data Analysis). Each section describes the QA/QC procedures that apply to that element of the Benthic Program. The types and quantity of data needed and a description of how the data will be acquired to support the program's objectives are presented in Section 4.0 (Field

Program). Two types of data are needed: fixed site and probability-based. This section specifies the sampling season, site selection process, sampling boundaries, site acceptance criteria, and sample acceptance criteria. A description of how the samples will be handled and analyzed in the laboratory is presented in Section 5.0 (Laboratory Processing). Laboratory QA/QC procedures, data precision, and performance criteria are summarized in this section and described in detail in Attachment 1 (Versar's Laboratory Standard Operating Procedures Manual). Enumeration accuracy is addressed in this section. Although the accuracy of identifications cannot be truly tested, accuracy is approximated by consultation with taxonomic experts and the use of voucher collections, which are available to and shared among laboratories. Data management, the procedures used to minimize data entry errors, and the limits of errors, are described in Section 6.0 (Data Management). Finally, the intended use of the data, data analysis methods, annual estimates, the precision of estimates, and the reporting procedures, are described in Section 7.0 (Data Analysis).

3.0 PROGRAM ORGANIZATION, MANAGEMENT, AND PERSONNEL

The organizational framework for the study, areas of responsibility of program personnel, lines of communication with the Department of Natural Resources, and relevant experience of the scientific and technical staff are described briefly. The specific staff for each area of responsibility are named in Section 3.9.

3.1 PROJECT MANAGER

The Project is organized with a Project Manager responsible for all day-to-day activities. The Project Manager is responsible for all administrative and technical matters and is the liaison between the Maryland Department of Natural Resources (MD DNR) and Versar. The Project Manager manages all subcontracts. He/she directs the Quality Assurance/Quality Control Program and is responsible for all reports and data produced for MD DNR. The Project Manager is also the point of contact for technical liaison with the U.S. EPA Chesapeake Bay Program, the Virginia Department of Environmental Quality, the Virginia Chesapeake Bay Benthic Monitoring Program, and any other external person or group, to further MD DNR objectives.

The Project Manager functions through seven Activity Managers each responsible for different aspects of the program. These Activity Managers are: (1) Lead Scientist, coordinating on sampling design, data analysis, interpretation, and reporting, (2) QA Manager, (3) Field Operations Chief, (4) Laboratory Manager, (5) Data Manager, (6) GIS Coordinator, and (7) Document Production Manager.

3.2 LEAD SCIENTIST

The Lead Scientist provides coordination, and technical and scientific support on sampling design, data analysis, interpretation, and reporting.

3.3 QUALITY ASSURANCE (QA) MANAGER

The QA Manager is responsible for ensuring the implementation of all the Quality Assurance/ Quality Control (QA/QC) procedures. He/she verifies that the QA/QC protocols and standards are applied to all work to assure that the results obtained are of the type and quality needed and expected. The QA Manager is responsible for maintaining the official, approved QA Project Plan. The QA Manager works closely with the Field Operations Chief and the Data Manager, and reviews field sampling plans and QA/QC data outputs. The QA Manager also serves as Laboratory Manager, overseeing day-to-day operation of the Laboratory QA/QC Program for Versar and subcontractor laboratories.

3.4 FIELD OPERATIONS CHIEF

The Field Operations Chief is responsible for all field activities, equipment, and crew. He/she works closely with the Program Manager and other Activity Managers. Based on directives from the Program Manager, he/she identifies activities and sites "piggy-backing" on the "normal" project scope and works with the GIS coordinator and Data Manager to prepare for sampling. The Field Operations Chief functions as Chief Scientist during sampling cruises, coordinating with the vessel captain, ensuring the correct functioning and operation of all instruments and gear, and supervising all other scientific staff. After the cruise, the Field Operations Chief provides data to the Data Manager and samples to the Laboratory Manager.

3.5 LABORATORY MANAGER

The Laboratory Manager is responsible for all samples and data produced in the laboratory of Versar or any subcontractor. He/she provides samples to subcontractors when necessary, and works with the Data Manager, subcontractors, and laboratory staff to ensure that sample tracking systems, sample processing, data sheets, and data entry meet all quality standards. Because most laboratory activities (sample handling, document and custody, data generation) are closely associated with the QA/QC Program, the Laboratory Manager is also the QA Manager.

3.6 DATA MANAGER

The Data Manager is responsible for data logging, reduction, and transmittal. He/she works closely with the QA Manager to ensure that data meet data quality objectives and to minimize the possibility of errors. Working with the GIS Coordinator and Field Operations Chief, the Data Manager produces site lists, field data sheets, and sample labels prior to sampling. Once field data are downloaded, the Data Manager activates the sample tracking system and prints laboratory data sheets. Once data are generated by the laboratories, the Data Manager reconciles them with the sample tracking system and subjects them to extensive checking and quality control under the direction and scrutiny of the QA Manager. Finally, the Data Manager adds these data to the long-term benthic data base and produces routine data analyses.

3.7 GIS COORDINATOR

The GIS coordinator assists in site selection and visualization prior to sampling, and presentation of results after data have been generated and analyzed. Working with the Field Operations Chief, he/she selects spatially random sites and prepares maps of all

sampling sites to facilitate field operations. Once data analysis is complete, he/she produces graphics to depict MD DNR's results.

3.8 DOCUMENT PRODUCTION MANAGER

The Document Production Manager assists in report production. He/she supervises the document production staff and works with the Program Manager and project technical staff to produce reports for MD DNR.

3.9 SUBCONTRACTORS

Cove Corporation of Lusby, Maryland, provides to Versar the taxonomic expertise of Ms. Nancy Mountford and Mr. Tim Morris. Freshwater Benthic Services, Inc. (FBS), provides to Versar the taxonomic services of Mr. Michael Winnell on a limited basis. Versar closely monitors the QA/QC protocols of its subcontractors and ensures and verifies that these protocols are similar to those of Versar's. The Laboratory and QA Manager is responsible for any work of Versar's subcontractors. Versar's technical and managerial interactions with Cove Corporation are facilitated by proximity of locations and electronic communications, as well as frequent past working relationships. Cove Corporation processes benthic samples for Versar on an as needed basis.

3.10 TECHNICAL STAFF FOR EACH AREA OF RESPONSIBILITY, QUALIFICATIONS

Mr. David Wong, Project Manager (B.S., Marine Biology, University of Maryland, 1999), serves in leadership roles for several marine and tidal freshwater benthic surveys and water quality monitoring projects at Versar. Mr. Wong has extensive experience in benthic sample collection, fisheries sampling techniques, laboratory management, benthic habitat mapping, and data interpretation and reporting. His experience extends from New England to Georgia, the Gulf Coast, and Guantanamo Bay Cuba in settings that include the nearshore, ocean inlets, coastal bays, and estuarine bays and tributaries. Mr. Wong's project duties include project management, data management, reporting, and stakeholder interaction. Mr. Wong has gained extensive experience with fiscal and administrative tasks, as well as with coordination between field operations, laboratory operations, data analysts, and client contract managers.

Dr. Roberto Llansó, Lead Scientist, is Versar's Principal Scientist in estuarine and coastal ecology. He is responsible for Versar's benthic ecology projects and has expertise in the development and application of biological criteria in estuaries. Dr. Llansó has managed the Chesapeake Bay Long-Term Benthic Monitoring Program (LTB) for MD DNR from 1999 to 2020. He has participated in the development, evaluation, and update of the Chesapeake Bay Benthic Index of Biotic Integrity and has developed similar indices for EPA's Mid-Atlantic Integrated Assessment (MAIA) and New York DEC's Hudson River Estuary Program. From 1994 to 1999 Dr. Llansó led the Puget Sound Sediment Monitoring Program, where he was responsible for overall organization and implementation, including study design; development of field, laboratory, and analytical procedures; data collection, data analysis and interpretation; the management of program contracts; preparation of reports; and presentation of findings at management and scientific meetings. This program has collected sediment chemistry, toxicity, and benthic data at fixed and random locations in Puget Sound since 1989. Among other activities, Dr. Llansó provided technical support and expertise in the development of biological criteria in Puget Sound. Dr. Llansó holds a Ph.D. from the College of William and Mary, Virginia Institute of Marine Science, where he conducted research on the effects of low dissolved oxygen on benthic communities in the Chesapeake Bay. He has gained considerable marine taxonomic experience throughout United States. Dr. Llansó is particularly interested in taxonomic standardization issues for which he founded and incorporated the Northern Association of Marine Invertebrate Taxonomists (NAMIT).

Ms. Suzanne Arcuri is Laboratory and QA Manager since January 2017. Ms. Arcuri (B.S. Biology, Pennsylvania State University, 1987) has worked as a Senior Taxonomist for Versar for 16 years. Ms. Arcuri supervises Versar benthic and sediment laboratories; assigns samples for sorting, taxonomy, and sediment analysis; and is responsible for performing and documenting sorting and taxonomic QA/QC procedures in Versar's laboratory. Ms. Arcuri has 31 years of experience in the identification of estuarine and marine benthic organisms from the East Coast of the U.S, and has extensive experience at training technical personnel to process benthic samples. Ms. Arcuri has been responsible for the taxonomic identification of thousands of estuarine and coastal benthic samples collected from Rhode Island, New York, New Jersey, Delaware, Maryland, Virginia, North Carolina, and Georgia, as well as California and Texas for a U.S. Maritime Administration contract. Ms. Arcuri has been tasked by U.S. EPA to perform QA/QC taxonomic re-identifications and re-enumerations for samples collected as part of National Coastal Condition Assessment surveys. These samples were collected from various regions along the Atlantic and Gulf coasts of the U.S.

Mr. Patrick Donovan, Field Operations Chief, has a Bachelor of Science degree in Marine Biology by The College of Charleston, and four years of experience at Versar that includes benthic sampling. He has participated in LTB for three full field seasons, has received training from Kathy Dillow (previous Field Coordinator) and has extensive knowledge and familiarity with Chesapeake Bay. Mr. Donovan's qualifications include operating and maintaining boats, conducting benthic and fish sampling using a variety of

sampling gears, deploying water quality sondes, storm water monitoring, stream benthic sampling and habitat assessment, and stream geomorphological assessments.

Mr. Michael Lane (M.S. Biological Sciences, Old Dominion University, 1991), Data Manager, has 30 years of experience as data analyst at Old Dominion University Department of Biological Sciences. He conducts and interprets statistical analyses of ecological data sets, literature searches, and develops tables, charts and graphics for technical reports and publications. His duties at ODU include statistical and graphic analysis of Chesapeake Bay Program data and collaboration in technical reports and scientific publications. The Data Manager maintains the LTB database and performs statistical analyses of the data under the direction of the Lead Scientist.

Ms. Allison Brindley, Geographic Information Systems (GIS) Coordinator, has over 30 years of experience as an environmental scientist, specializing in database management and spatial analysis and interpretation using GIS. Ms. Brindley's expertise is designing and applying GIS tools to analyses of current and potential future ecological conditions and devising plans for assessing restoration potential. As the primary GIS analyst at Versar's, she is responsible for the integral aspects of spatial and temporal analysis and graphical representation of data. She is also part of a modeling team for watershed assessments and stormwater tracking projects, providing analyses of point and non-point pollution, thermal and chemical discharge, cumulative urban effects, and flow-related impacts. For LTB, Ms. Brindley implements the GIS procedures for the random site allocations and provides graphic displays of all data.

Ms. Nancy Mountford and Mr. Tim Morris of Cove Corporation are recognized authorities on the taxonomy and identification of Chesapeake Bay benthic organisms. Cove Corporation has been on Versar's Team for a variety of projects for many years. They have participated in power plant impact studies on benthic biota, including studies of meiobenthic species, and have collaborated with LTB since the program's inception. Ms. Mountford was a senior research assistant on benthic field programs at Calvert Cliffs between 1971 and 1978 and received a Master of Science degree in Zoology from the University of Maryland in 1984. Mr. Morris received a Master of Science degree in Biology from Old Dominion University in 1986.

4.0 FIELD PROGRAM

The field program is supervised by the Field Operations Chief and consists of four phases of activity involving all types of sampling: (1) site selection, (2) cruise preparation, (3) sampling cruise, and (4) post-cruise. Samples are collected once each year in summer at random and fixed locations. Samples were collected in spring at fixed locations but the spring sampling was discontinued in 2009.

Three seasonal definitions are used by the program (Table 4-1). The broadest, least restrictive, Chesapeake Bay definition is shared with the Virginia Benthic Monitoring Program and the Chesapeake Bay benthic index of biotic integrity; only data meeting this definition are analyzed. The intermediate, more restrictive, Maryland definition is inclusive of all Maryland data used for seasonal trend analysis at historic (fixed) sites sampled since 1984; every effort is made to collect samples within this time window each year. The most restrictive "target" definition is a two-week period including approximately 60-70% of the Maryland data; sample collection occurs in this period each year and, if logistically feasible, all sampling is completed during this window.

Table 4-1. Season definitions. Spring: Fixed sites only, discontinued in FY 2009. Summer: Fixed and probability sites.

Season	Chesapeake Bay	Maryland	Target
Spring	16 April - 15 July	22 April - 27 May	07 - 20 May
Summer	16 July - 30 Sept	29 July - 30 Sept	03 - 16 Sept

4.1 SITE SELECTION

Three types of sites are sampled by the program: fixed, probability, and special sites.

4.1.1 Fixed Sites

The 27 fixed sites (Figure 4-1) are used to identify temporal trends in benthic condition. Most of the sites have been sampled since 1984 (Figure 4-1). They are all sampled summer, and they have been sampled in spring through 2008. Sites are defined by geography (within 1 km from a fixed location) and by specific depth and substrate criteria. Table 4-2 is a list of the 27 fixed sites and their criteria.

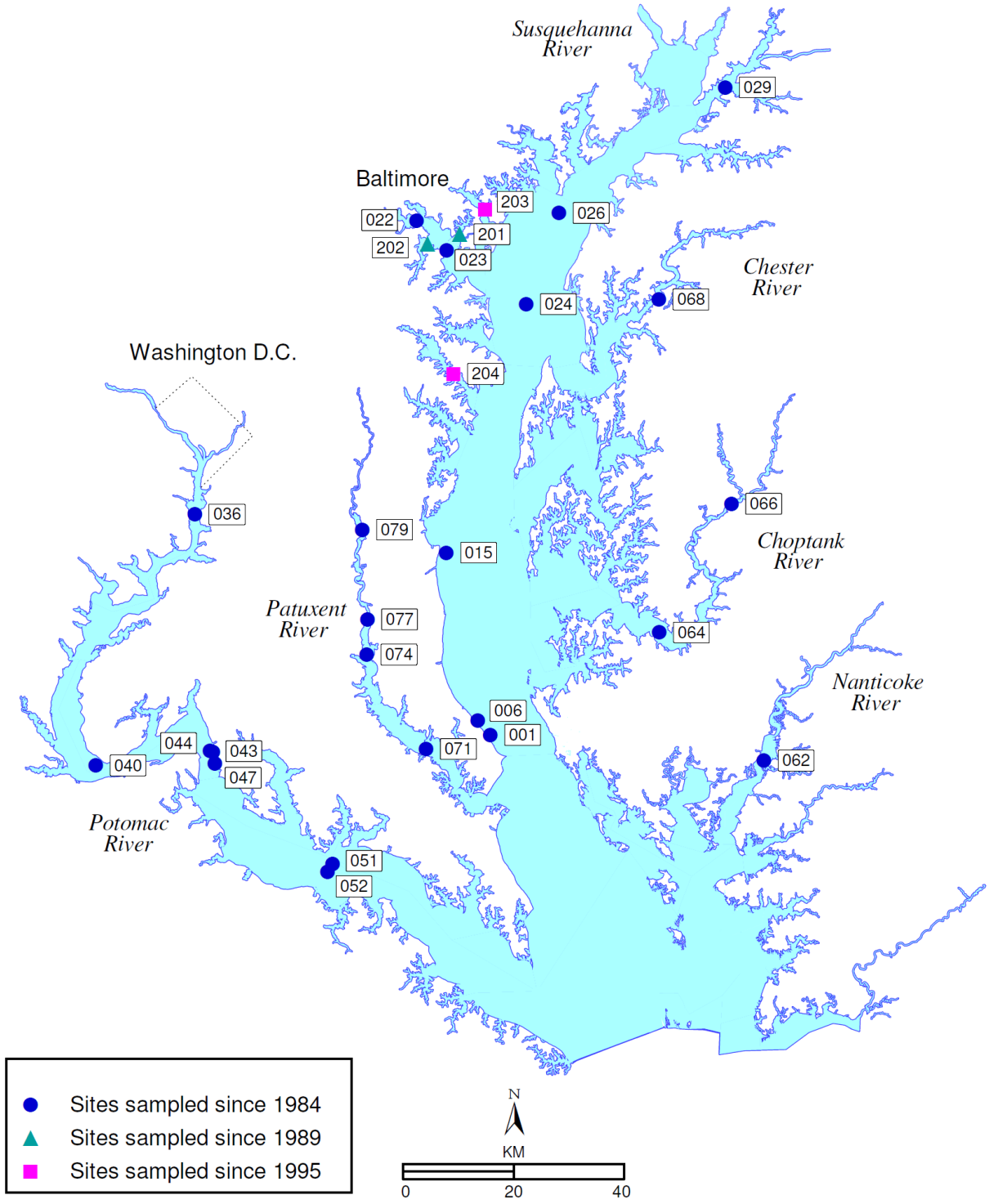

Figure 4-1. Maryland fixed benthic sites

Table 4-2. Location, habitat (Table 5, Weisberg et al. 1997), sampling gear, and habitat criteria for fixed sites. *Station 022 relocated across the channel during the 2010 field season because of construction in the old site.

Stratum	Sub-Estuary	Habitat	Station	Latitude WGS84)	Longitude (WGS84)	Sampling Gear	Habitat Criteria		
							Depth (m)	Siltclay (%)	Distance (km)
Potomac River	Potomac River	Tidal Freshwater	036	38.769788	-77.037534	WildCo Box Corer	<=5	>=40	1.0
		Oligohaline	040	38.357466	-77.230537	WildCo Box Corer	6.5-10	>=80	1.0
		Low Mesohaline	043	38.384479	-76.988329	Modified Box Corer	<=5	<=30	1.0
		Low Mesohaline	047	38.363825	-76.983737	Modified Box Corer	<=5	<=30	0.5
		Low Mesohaline	044	38.385633	-76.995698	WildCo Box Corer	11-17	>=75	1.0
		High Mesohaline Sand	051	38.205355	-76.738622	Modified Box Corer	<=5	<=20	1.0
		High Mesohaline Mud	052	38.192304	-76.747689	WildCo Box Corer	9-13	>=60	1.0
Patuxent River	Patuxent River	Tidal Freshwater	079	38.750457	-76.689023	WildCo Box Corer	<=6	>=50	1.0
		Low Mesohaline	077	38.604461	-76.675020	WildCo Box Corer	<=5	>=50	1.0
		Low Mesohaline	074	38.548962	-76.676186	WildCo Box Corer	<=5	>=50	0.5
		High Mesohaline Mud	071	38.395132	-76.548847	WildCo Box Corer	12-18	>=70	1.0

Table 4-2. (Continued)

Stratum	Sub-Estuary	Habitat	Station	Latitude (WGS84)	Longitude (WGS84)	Sampling Gear	Habitat Criteria		
							Depth (m)	Siltclay (%)	Distance (km)
Upper Western Tributaries	Patapsco River	Low Mesohaline	023	39.208283	-76.523354	WildCo Box Corer	4-7	>=50	1.0
	Middle Branch	Low Mesohaline	022*	39.258082	-76.59512	WildCo Box Corer	2-6	>=40	1.0
	Bear Creek	Low Mesohaline	201	39.234167	-76.497501	WildCo Box Corer	2-4.5	>=70	1.0
	Curtis Bay	Low Mesohaline	202	39.217839	-76.564171	WildCo Box Corer	5-8	>=60	1.0
	Back River	Oligohaline	203	39.275005	-76.444508	Young-Grab	1.5-2.5	>=80	1.0
	Severn River	High Mesohaline Mud	204	39.006954	-76.504955	Young-Grab	5.7-7.5	>=50	1.0
Eastern Tributaries	Chester River	Low Mesohaline	068	39.132509	-76.078780	WildCo Box Corer	4-8	>=70	1.0
	Choptank River	Oligohaline	066	38.801455	-75.921827	WildCo Box Corer	<=5	>=60	1.0
		High Mesohaline Mud	064	38.590459	-76.069331	WildCo Box Corer	7-11	>=70	1.0
	Nanticoke River	Low Mesohaline	062	38.383960	-75.849990	Petite Ponar Grab	5-8	>=75	1.0

Table 4-2. (Continued)

Stratum	Sub-Estuary	Habitat	Station	Latitude (WGS84)	Longitude (WGS84)	Sampling Gear	Habitat Criteria		
							Depth (m)	Siltclay (%)	Distance (km)
Upper Bay	Elk River	Oligohaline	029	39.479505	-75.944836	WildCo Box Corer	3-7	>=40	1.0
	Mainstem	Low Mesohaline	026	39.271450	-76.290013	WildCo Box Corer	2-5	>=70	1.0
		High Mesohaline Mud	024	39.122004	-76.355673	WildCo Box Corer	5-8	>=80	1.0
Mid Bay	Mainstem	High Mesohaline Sand	015	38.715126	-76.513679	Modified Box Corer	<=5	<=10	1.0
		High Mesohaline Sand	001	38.419001	-76.418385	Modified Box Corer	<=5	<=20	1.0
		High Mesohaline Sand	006	38.442000	-76.444261	Modified Box Corer	<=5	<=20	0.5

4.1.2 Probability Sites

Probability sites are used to assess the extent of the Maryland Bay that meets the Chesapeake Bay Benthic Community Restoration Goals (Ranasinghe et al. 1994, updated by Weisberg et al. 1997; Alden et al. 2002) each year. A fresh set of 150 sites are selected at random each year and sampled. They are sampled only in summer because the restoration goals have only been set for summer.

Probability sites are allocated according to a stratified random sampling scheme designed to produce an annual estimate with known precision of the tidal area meeting the restoration goals for the Maryland Bay, as well as estimates for six subdivisions or strata. Samples are allocated equally among strata (Figure 4-2, Table 4-3). Regions of the Maryland mainstem deeper than 12 m are not included in the sampling strata because these areas are subjected to summer anoxia and have consistently been found to be azoic. Except for these excluded areas (Deep Mainstem, Figure 4-2), every point of the Maryland Bay tidal bottom deeper than 1 m mean lower low water (MLLW) has a chance of being sampled.

Table 4-3. Allocation of probability-based baywide samples, in and after 1995. Maryland areas exclude 676 km^2 of mainstem habitat deeper than 12 m

State	Stratum	Area			Number of Samples
		km^2	State %	Bay %	
Maryland	Deep Mainstem	676	10.8	5.8	0
	Mid Bay Mainstem	2,552	40.9	22.0	25
	Eastern Tributaries	534	8.6	4.6	25
	Western Tributaries	292	4.7	2.5	25
	Upper Bay	785	12.6	6.8	25
	Patuxent River	128	2.0	1.1	25
	Potomac River	1,276	20.4	11.0	25
	TOTAL	6,243	100.0	53.8	150

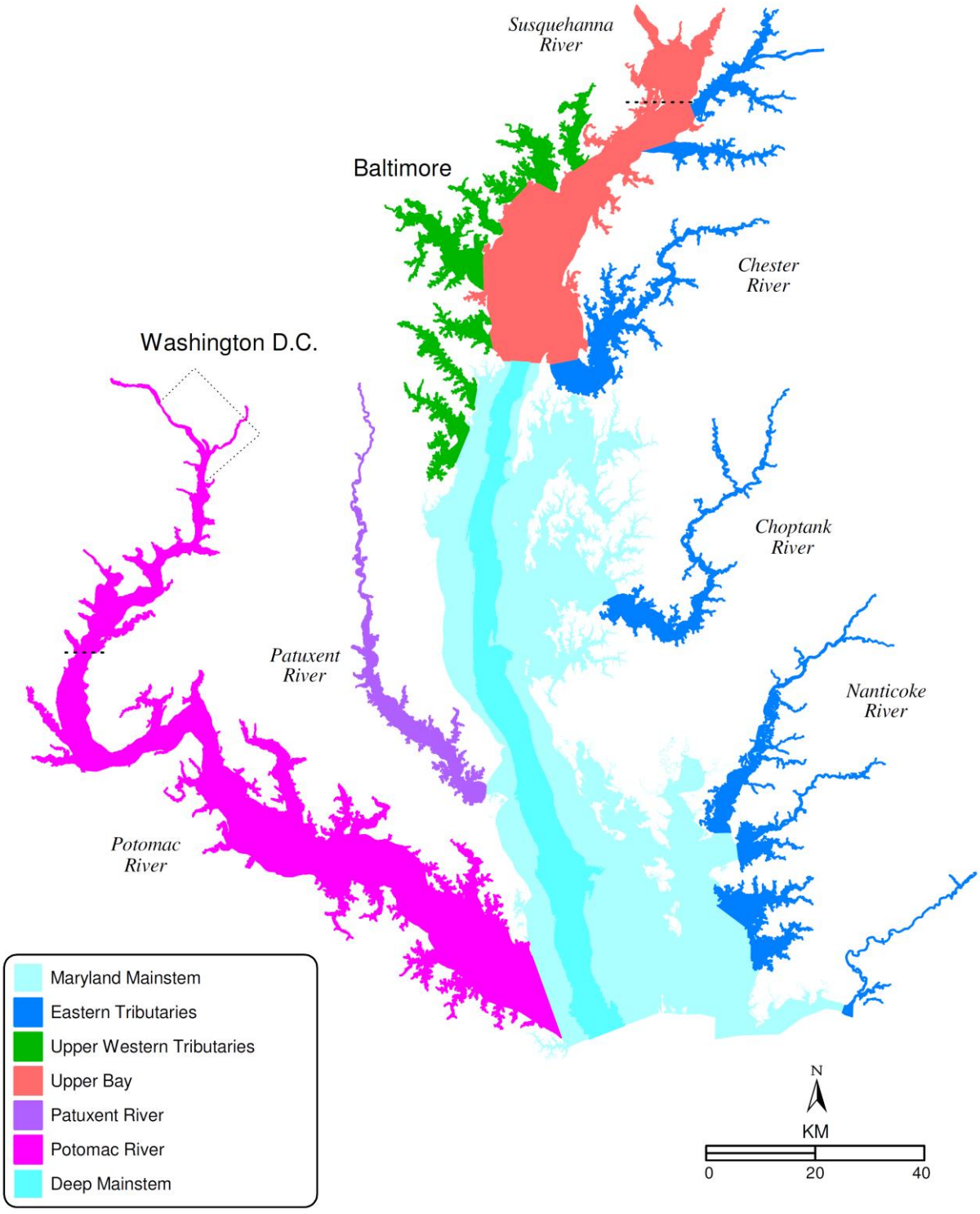

Figure 4-2. Maryland baywide sampling strata

4.1.2.1 Sampled Area Definition

The primary requirement for comparability of annual "healthy" area estimates among years is that estimated area boundaries be constant. Stratum definitions and sample allocation schemes may be altered provided the same area is covered. Although the precision of the estimate may change depending on the nature and magnitude of the stratification changes, estimates will be comparable from year to year.

Although some boundaries of the Maryland Bay are clear, others are poorly defined. Jurisdictional boundaries such as the Washington D.C.-Maryland line in the Potomac and the Virginia-Maryland line dividing the Chesapeake Bay, Tangier Sound, and Pocomoke Sound are clear. However, sampling limits on Bay and tributary margins are most often controlled by practical considerations such as the draft of the sampling vessel. The upstream distance sampled in tributaries is often subjective because heads of tide are not well known.

The purpose of this section is to define LTB sampling area boundaries for these poorly defined margins. Definitions are provided for Bay margins at the land-water interface, and for each of the 42 tidal tributaries of Chesapeake Bay.

4.1.2.2 The Land-water Interface at Bay and Tributary Margins

The Maryland Long-Term Benthic Monitoring and Assessment Program samples all bottom areas of the Chesapeake Bay and its tidal tributaries deeper than 1 m MLLW. MLLW is the most prevalent datum in use. It is the 19-year mean for the lower of the two daily low-tides occurring in areas with semi-diurnal tides, such as the Chesapeake Bay.

All tidal bottom areas are subject to sampling except for areas that may be restricted by the government, such as bay bottom adjacent to the Aberdeen Proving Grounds and the Bloodsworth Island US Naval Reservation. Navigation charts warn of unexploded ordinance in these areas which, therefore, are unsuitable for benthic sampling. On a smaller scale, cable and pipeline areas designated on nautical charts are also avoided.

4.1.2.3 Tributary Head Sampling Limits

The LTB objective is to sample as far up each tributary as the uppermost point at which tidal influences occur ("head of tide") or as close to it as possible. Accordingly, the farthest point sampled up each tributary is the head of tide, or the navigable limit according to nautical charts, which ever is closer to the Bay.

A two-step process was used to identify sampling limits for rivers with tidally influenced lower segments which drain into the Chesapeake Bay. Heads of tide and limits

of navigability were determined and the sampling limit was set accordingly. The results are presented in Table 4-4. By our criteria, determinations were required for 36 of the 42 rivers identified by the State of Maryland.

Heads of tide were determined using the MD DNR's tidal wetland maps. These maps delineate wetland areas on a background aerial photograph. For all tributaries where heads of tide were delineated, they were identified as marked. Otherwise, the limit was judged to be at the point of the uppermost delineated tidal wetland.

Limits of navigability were identified from nautical charts. For some tributaries, navigation is not possible because heads of tide are beyond the limits of the nautical charts. In these cases, the sampling limit was defined as the uppermost point that can be safely navigated based on information from nautical charts or other sources. The results are presented in Table 4-4.

4.1.2.4 Probability Site Selection Process

To ensure that 25 samples are collected at random, 30 sites are selected for sample collection as follows:

1) For each stratum, the GIS Coordinator selects up to 1,000 points at random in a uniform distribution from an area that is a superset of the stratum, using the program written specially for the purpose. Decimal degree reference coordinates are used with a precision of 0.000001 degrees (approximately 1 meter) which is a smaller distance than the accuracy of positioning; therefore, no area of the bay is excluded from sampling and every point in the Maryland Bay has a chance of being sampled.

2) The GIS image of the stratum is overlayed on the selected points and points on land are eliminated.

3) The first 50 selected points are plotted on navigation chart look-alikes and provided to the Field Operations Chief together with a list of coordinates.

4) The Field Operations Chief eliminates any of these points which either (a) are in prohibited areas, (b) are clearly shallower than 1 m MLLW, (c) are close to submerged cables or other obstacles, or (d) cannot be approached because of intervening shallow waters. If less than 30 sites remain after this process, additional sites are plotted until 30 sites are selected.

5) Thirty potential sampling sites are now available in each stratum. The selection order of each site is known and stored along with the coordinates.

Table 4-4. Heads of tide, benthic sampling limits, and the distance between them for tidal rivers draining into the Maryland Chesapeake Bay. Reasons for difference between head of tide and sampling limit: A - Sampling limit is at jurisdictional boundary; B - Unable to navigate; C - Navigational information not available.

River	Head of Tide	Sampling Limit	Distance (km)	Reason
Potomac	Little Falls Dam	DC - MD line	20	A
Port Tobacco	State Route 6	Warehouse Point	2	B
Wicomico	State Route 234	Newport Run	6	B
St. Mary's	State Route 5	Tippety Witchity Island	4	B
Patuxent	State Route 214	State Route 4	10	C
West	State Route 468	Smith Creek	2	B
South	Rutland Road	US Route 50/301	2	B
Severn	US Route 97	Indian Landing	2	B
Magothy	Catherine Avenue	Magothy Bridge Road	2	B
Patapsco	US Route 695	Hanover Street Bridge	6	B
Back	Redhouse Creek	US Route 695	2	B
Middle	State Route 150	Head of tide	0	-
Gunpowder	US Route 40	Iron Point	5	B
Bush	US Route 40	Bush Point	1	B
Susquehanna	Robert Island	Spencer Island	1	C
Northeast	State Route 272	Stony Run	1	B
Elk	State Route 7	Locust Point	6	B
Bohemia	Telegraph Road	Labbide Mill Creek	4	C
Sassafras	US Route 301	Wilson Point	5	B
Chester	State Route 313	State Route 290	7	B
Corsica	State Route 213	Sycamore Point	2	B
Wye	US Route 50	Sportsmans Neck	3	B

Table 4-4. (Continued)

River	Head of Tide	Sampling Limit	Distance (km)	Reason
Wye East	Wye Mills - Queen Anne Road	2 km upstream of Wye Landing	5	B
Miles	Potts Mill Creek	Unnamed creek near Todds Corner	3	B
Tred Avon	State Route 33	Easton Point	1	B
Choptank	State Route 313	Forge Branch	4	B
Little Choptank	Cambridge-Hudson Road	Lee Creek	2	B
Blackwater	All tidal	Maple Dam Road	18	C
Transquaking	Drawbridge Road	Head of tide	0	-
Chicamacomico	US route 50	Head of tide	0	-
Honga	All tidal	Keenes Point	NA	B
Nanticoke	US Route 13	MD-DE state line	10	A
Wicomico	Tony Tank Creek	Head of tide	0	-
Manokin	US Route 13	Locust Point	10	B
Big Annemessex	State Route 413	Persimmon Point	5	B
Pocomoke	Whiton Crossing Road	Snow Hill	15	C

4.1.3 Special Sites

Special sites are not associated with the core benthic monitoring program, but rather with special projects that have special objectives and that take advantage of this program to collect samples economically and with simplified logistics. The sites may be additional ones which otherwise would not have been sampled, or involve additional sampling or data collection at regularly sampled sites, or a combination of both. The specifics vary from year to year and are governed by each special project.

4.2 CRUISE PREPARATION

There are several aspects of cruise preparation. They are (1) vessel, crew, and scientific party scheduling, (2) site identification, (3) label and field data sheet production, and 4) equipment coordination.

4.2.1 Vessel, Crew, and Scientific Party Scheduling

Large and small vessels are used by the Maryland Long-Term Benthic Monitoring and Assessment Program and scheduling is specific to each type. Based on the geographic distribution of sampling points and compromises between convenience, cost, ability to withstand weather, availability of boat ramps, and speed in and accessibility of shallow waters, the Field Operations Chief allocates sites for sampling from large and small vessels. Allocations are flexible, and usually evolve as sampling progresses.

4.2.1.1 Large (University of Maryland) Vessels

Reservations for these vessels are typically made six months or more in advance, and the Field Operations Chief coordinates scientific party, vehicle, and trailer rendezvous from Columbia and vessel loading and departure from Solomons with the boat captain.

4.2.1.2 Small (Versar) Vessels

The Field Operations Chief coordinates scientific party, crew, vehicle, vessel and trailer availability, rendezvous, and loading in Columbia.

4.2.2 Site Identification

1) The GIS Coordinator passes a file containing the “top 30" probability site selections for each stratum (Section 4.1.2.4 above) to the Data Manager. The Data Manager provides each site with a five-digit station number. The first two

digits represent the year (1994=01, 1995=02, and so on; 2021=28). The third digit represents the stratum (1=Potomac, 2=Patuxent, 3=Western Tributaries, 4=Eastern Tributaries, 5=Mid-Bay Mainstem, and 6=Upper Bay). Within each stratum, the first 25 selected sites are numbered in sequential order from south to north, while sites 26-30 are numbered in selection order; sampling must be attempted at sites 1-25, while the Field Operations Chief may decide whether or not to collect extra samples based on progress up to that point. Twenty-five samples are processed from each stratum each year; symmetry of sampling frequency among strata and among years considerably simplifies the mathematics of estimation.

2) The Data Manager combines the coordinates and list of fixed sites and any special sites with the list of 180 probability sites, and assigns sample serial numbers and any other necessary variables, creates a list of sampling sites for the Field Operations Chief including sampling gear and other pertinent information, and provides an electronic file to the GIS coordinator.

3) The GIS coordinator produces a set of navigation chart look-alikes with a comprehensive plot of site locations. The Field Operations Chief plots these points on actual navigation charts. A fresh set of Chesapeake Bay charts is purchased annually for this purpose.

4.2.3 Label and Field Data Sheet Production

The Field Operations Chief and Data Manager coordinate to produce sample labels, data sheets, and any other necessary or desirable paperwork electronically.

4.2.4 Equipment Coordination

The Field Operations Chief ensures that all necessary instruments, sampling gear, and equipment are available and in good working order. All instruments are calibrated on a regular basis.

4.3 SAMPLING CRUISE

4.3.1 Station Location

Stations are located using a differential Global Positioning System accurate to within 10 m. The WGS84 coordinate system (practically equal to NAD83) is currently used.

At fixed sites where depth and habitat type have been defined (Table 4-2), the Field Operations Chief verifies that parameters are within permissible ranges in addition to the location being correct. If parameters vary beyond acceptable ranges, the boat is repositioned until long-term habitat criteria are met.

4.3.2 Sampling Failure

At probability sites, it may not be possible to collect a benthic sample for several reasons: (1) intervening shallow water may be an obstacle to reaching a site, (2) a site may be too shallow for navigation, (3) the nature of bottom sediments (oyster reef or shell-hash) may prevent grab closure, and (4) failure of navigation or hydrographic instrumentation may result in loss of ancillary data. In the case of (1) and (2), sampling will be attempted at least once by small boat before the site is discarded. In the case of (3) three attempts at relocation will be made within a 37 m circle, and three additional attempts within a 37-100 m distance from the original point in different directions. If an acceptable sample cannot be collected, the site will be discarded. In the case of (4), the site will be resampled after equipment is repaired. Only in extreme circumstances where overall success of the program is jeopardized, can a sample be substituted for logistical reasons. An example would be dropping a single sample six hours travel time up a tributary, collection of which threatened to prevent sampling several other sites because the "end of summer" deadline was approaching.

4.3.3 Water Column Measurements

At fixed sites, water column vertical profiles of temperature, conductivity, salinity, dissolved oxygen concentration (DO), and pH are measured using a YSI EXO2 Sonde or Hydrolab DataSonde 4a. The profiles consist of water quality measurements at 1 m intervals from surface to bottom at sites 10 m deep or less, and at 2 m intervals, with additional measurements at 1.5 m intervals in the vicinity of the pycnocline, at sites deeper than 10 m. At all other sites, surface and bottom measurements are made. Table 4-5 lists the measurement methods.

All instruments are checked for required maintenance and calibrated against accepted and reasonable standards prior to and after each cruise and routinely during extended periods of field (or lab) use. For example, on the ~16 day Chesapeake Bay cruise, the YSI or Hydrolab is recalibrated every other day. The instrument is also recalibrated before and after each cruise to determine the amount of drift. The sondes are calibrated according to manufacturer's specifications, using the standard salinity and pH solutions supplied by the manufacturer, and the corrections recommended by the manufacturer's instructions. Specifications include air-saturation calibration of the DO probe and standard reference or buffer solution calibration of the conductivity and pH probes. DO meter calibrations and notable field measurements are occasionally checked using standard Winkler titrations. Calibrations are conducted by the Field Operations

Chief or designate (usually a senior member of the field crew) and recorded in calibration sheets (see Attachment 2) maintained in a central laboratory location.

Table 4-5. Methods used to measure water quality parameters

Parameter	Method
Temperature	Thermistor attached to Hydrolab DataSonde 4a or YSI EXO2
Salinity and Conductivity	Hydrolab DataSonde 4a four graphite electrode cell (open-cell design) or YSI EXO2 four nickel electrode cell, with automatic temperature compensation
Dissolved Oxygen	Hydrolab DataSonde 4a membrane-design DO sensor, or YSI EXO2 optical sensor, with automatic temperature and salinity compensation
pH	Hydrolab DataSonde 4a or EXO2 combined glass pH and reference sensor, automatically compensated for temperature

Field crews know the expected ranges of water quality values for each fixed site from previous measurements and the literature. As new measurements are taken, they are reviewed for outlying or unexpected values so that possible problems with instrument function can be resolved immediately.

4.3.4 Benthic Samples

Samples are collected using four kinds of gear depending on the program element and habitat type. At fixed sites (Figure 4-1, Table 4-2), a modified box corer ("post-hole digger"), which samples a 250 cm^2 area to a depth of 25 cm, is used in the nearshore shallow sandy habitats of the mainstem bay and tributaries. A Wildco box corer, which samples an area of 220 cm^2 to a depth of 23 cm, is used in muddy habitats or deep-water (> 5m) habitats in the mainstem bay and tributaries. A Petite Ponar grab, which samples 250 cm^2 to a depth of 7 cm, is used at the fixed site in the Nanticoke River to be consistent with previous sampling in the 1980s. At the two fixed sites first sampled in 1995 and at all probability sites, a Young grab, which samples an area of 440 cm^2 to a depth of 10 cm, is used. Different types of gear are used at fixed sites because these sites are historical. Many of these sites have been sampled since the mid 1970s or early 1980s. They continue to be sampled with the same gear to be consistent with past sampling. (Note: all data are standardized to number of organisms and biomass per squared meter).

At each site, sample penetration depth is measured for all samples; Wildco and modified box cores penetrating less than 15 cm, and Young and Petite Ponar grabs penetrating less than 7 cm into the sediment are rejected and the site is re-sampled. Samples are not accepted until these penetration depth criteria are met. Grabs and box corers with overflowing surface sediment are also discarded and the site re-sampled.

Three samples are collected for benthic community analysis at each fixed site. One sample is collected at each probability site.

In the field, samples are sieved through a 0.5-mm screen using an elutriative process. Organisms and detritus retained on the screen are transferred into labeled jars and preserved in a 10% formaldehyde solution stained with Rose Bengal (a vital stain that aids in separating organisms from sediments and detritus). Figure 4-3 provides an overview of QA/QC for biological sample collection.

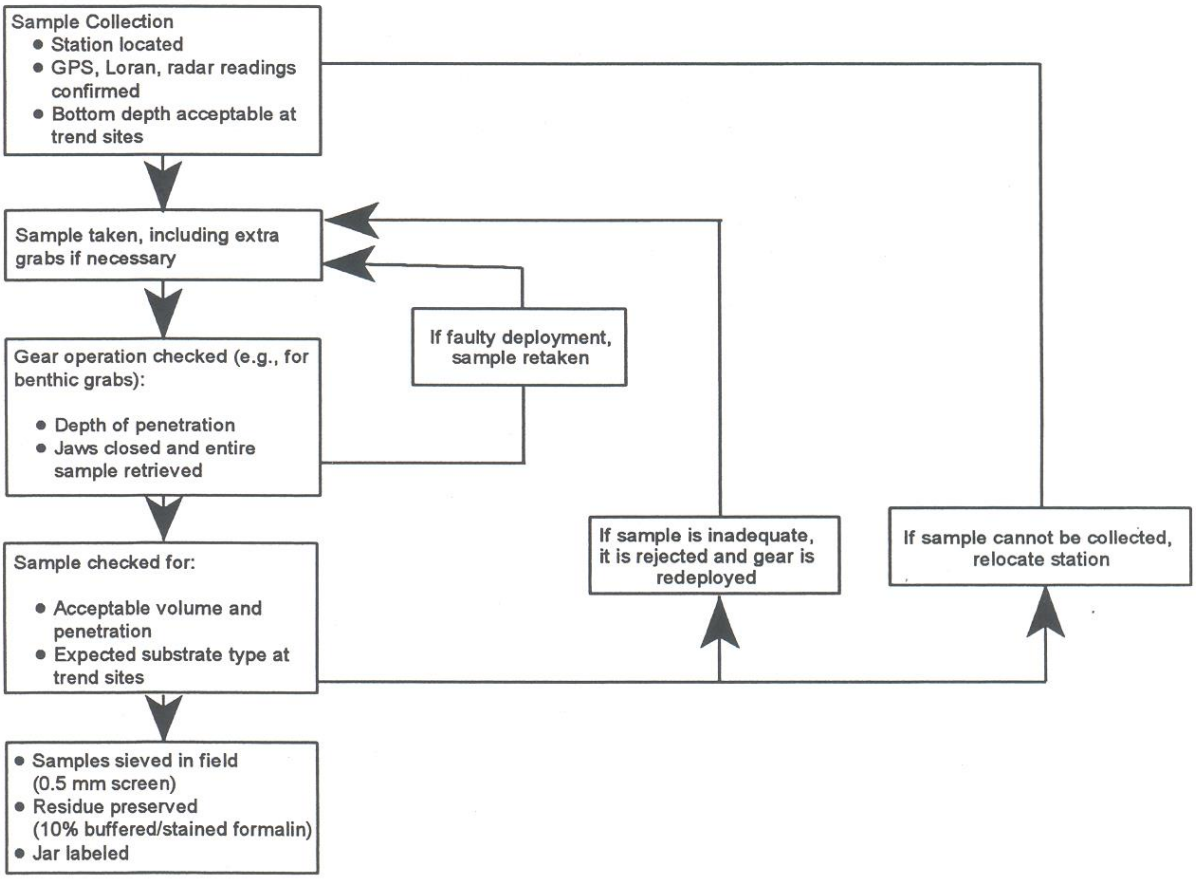

Figure 4-3. QA/QC for biological sample collection. See text for sampling failure and depth penetration criteria.

One surface-sediment (top 2-3 cm) sub-sample of approximately 120 ml is collected for grain-size, carbon, and nitrogen analysis from an additional grab sample at each site. This sub-sample is maintained in the dark on wet ice while on board, and frozen until processed in the laboratory. In addition, starting in summer 2021, three sediment sub-samples will be collected from a separate grab sample at each fixed site for benthic chlorophyll-a analysis. A 2.5 cm diameter syringe will be used to extract each

chlorophyll subsample. The syringe is pushed into the substrate, and a sediment core removed. The upper one centimeter of the core is extruded through a plexiglass ring 1 cm height x 2.5 cm diameter to precisely extract a slice of 4.91 m^3 of sediment which is preserved in a 50-ml centrifuge tube. Chlorophyll sub-samples are maintained in the dark on wet ice while on board, and frozen until processed by the contracts laboratory.

4.4 POST-CRUISE

All instruments are post-calibrated as described above (Section 4.3.3). Data are downloaded from the YSI or Hydrolab to computer files. Field sheets, field notes, and measurements on deck are entered into spreadsheets. Copies of all data files are transferred to the Data Manager.

The Data Manager generates a list of samples to be processed, including all fixed site samples, all special site samples, and the first 25 probability site samples in each stratum. The sample lists are imported into spreadsheets on the sample tracking computer in the benthic laboratory to begin the sample tracking process.

5.0 LABORATORY PROCESSING

Two types of samples, biological samples and sediment samples, are returned to the laboratory. Two types of data are produced for biological samples and five types of data for sediment samples. An overview of the biological sample processing QA/QC procedures is presented in Figure 5-1. Laboratory processing and the QA/QC procedures are described in detail in Versar's Laboratory Standard Operating Procedures (SOPs) Manual, which is included in Attachment 1 of this document.

5.1 BIOLOGICAL SAMPLES

Biological samples are tracked using serial numbers, chain of custody forms, electronic sample tracking logs, and data (bench) sheets. This information is used as applicable to track the location and progress of sample processing in the laboratories. Examples of all forms are provided in Attachment 2.

Prior to field sampling, each sample is given a unique serial number. This serial number is used to track the sample from field collection to delivery to the laboratory and through sample processing. For some projects, a sample tracking number is assigned at the time the sample is delivered to the laboratory. In the Long-Term Benthic Monitoring Program, sample serial numbers are assigned during the field preparation activities (see Section 4.2.2). A chain of custody form is associated with each incoming sample, and a electronic sample tracking log is used to track the processing stage of all samples, as described in Versar SOPs.

Benthic biological samples are processed to identify and enumerate each species present, and to measure species-specific ash-free dry weight biomass. Organisms are sorted from detritus under dissecting microscopes, identified to the lowest practical taxonomic level, and counted. Oligochaetes and chironomids are mounted on slides and examined under a compound microscope for genus and species identification. Counts are entered in data (bench) sheets.

Samples sorted by each technician are resorted on a regular but arbitrary basis to ensure that all organisms are removed from extraneous material. Approximately 10% of all samples processed are randomly selected and resorted for quality assurance. The minimum acceptable sorting efficiency is 90%, but typically efficiency of Benthic Program samples exceeds 95%. Any problems discovered during resorts result in review of recent and previous work which may also contain errors, additional training of technicians, and close supervision of technicians until performance is improved.

Species identifications are verified when organisms are transferred for biomass measurements. Samples sorted and identified by subcontractors are returned to Versar's lab for biomass determinations, which ensures an opportunity for verifying identifications

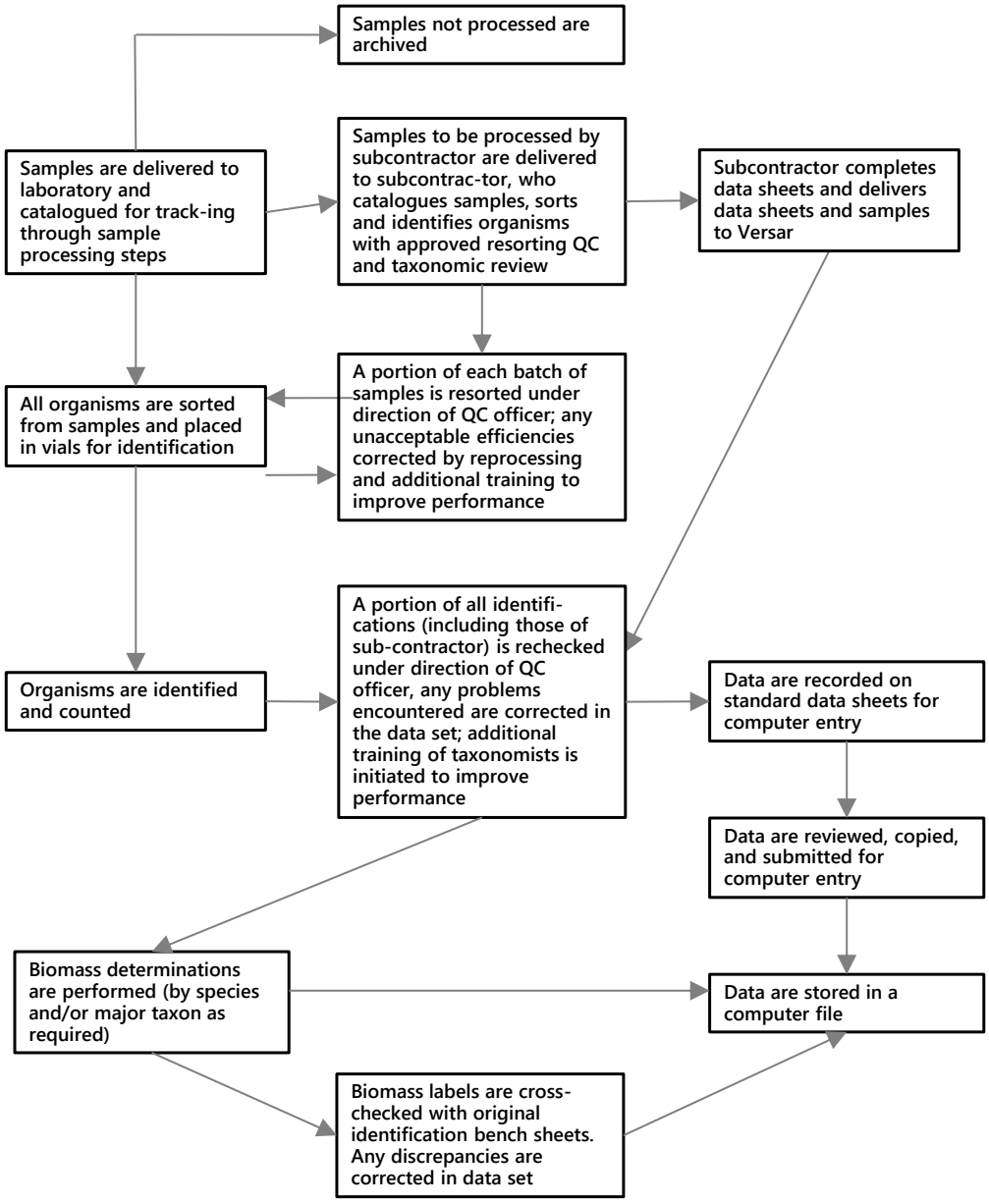

Figure 5-1. QA/QC for biological sample processing. Further detail is provided in Versar SOPs (Attachment 1).

and counts. A voucher collection containing representative specimens of each taxon identified is maintained by each laboratory. Questionable or unusual species identifications are confirmed by recognized experts in the appropriate taxonomic specialties. Contacts for taxonomic consultation include (but are not limited to) the Smithsonian Institution, the National Museum of Canada, and the Institute of Ocean Sciences. An extensive and current library of taxonomic and biological literature is available in-house for reference by technical specialists processing samples.

The QA Manager or an appointed representative recounts approximately 10% of all samples processed both internally and by subcontractors. Recounting is a method of evaluating both the performance of Versar personnel and subcontractors and the correctness of the recounted samples. The results from the resorting of samples, QC of identifications, and recounting, are recoded for each sample in QC sample resort and reidentification sheets, and logged electronically to produce a QA/QC submittal sheet (Attachment 2).

Ash-free dry weight biomass is measured directly for each species by drying the organisms to a constant weight at 60°C and ashing in a muffle furnace at 500°C for four hours and re-weighing (ash weight). The difference between dry weight and ash weight is the ash-free dry weight.

All laboratory balances are serviced annually by a specialized technician. Each balance is calibrated daily as required and balance efficiency is checked with standardized weights.

5.2 SEDIMENT SAMPLES

Silt-clay composition is determined from the sediment sub-sample, with a carbon and nitrogen analysis sample extracted and dried at the time of the silt-clay analysis.

For silt-clay determination, sand and silt-clay particles are separated by wet-sieving through a 63-μm stainless steel sieve followed by pipetting and weighing using standard procedures described in Plumb (1981) and Buchanan (1984).

Sediment sub-samples not immediately required for processing are frozen and stored to allow reprocessing for QC or confirmation of questionable results. Any questionable samples (i.e., values that fall outside of expected ranges, such as those listed in Table 4-2) are reprocessed for verification. Samples with percent silt-clay values within +/- 5% of the threshold (40%) used to classify sediments as mud or sand for the B-IBI, are reprocessed for verification.

Carbon and nitrogen content of dried sediments is determined using an elemental analyzer in accordance with the Standard Operating Procedure of the University of Maryland Chesapeake Biological Laboratory, Nutrient Analytical Services Laboratory.

Each sample is divided into two portions. One portion of the sample is used for Total Carbon (TC) determination, and the second portion is used for Total Inorganic Carbon (TIC) determination. No acid is applied. TC is determined by combustion at high temperature (975°C) in a carbon analyzer (Exeter Analytical, Inc., CE-440 Elemental Analyzer) and subsequent measurement of the carbon dioxide produced by thermal conductivity detection. Ashing of the second portion of the sample in a muffle furnace at low temperature (500°C) results in the removal of the organic carbon. The inorganic carbon remaining in the ash is then injected in the carbon analyzer and combusted at high temperature (975°C). The carbon produced during combustion is measured by thermal conductivity detection. Total Organic Carbon (TOC) is determined by subtracting the TIC results from the TC results. Total Nitrogen (TN) concentration is measured by thermal conductivity against a reference cell after all the carbon and hydrogen in the combustion chamber of the carbon analyzer is removed. Results are reported in percent.

5.3 QUALITY ASSURANCE/QUALITY CONTROL FOR SUBCONTRACTORS

The status of each sample processed by a subcontractor is tracked and recorded from the time the samples are received until the data sheets are delivered to Versar. Protocols have been established to ensure that all organisms are removed from sorted samples. Sorted material is retained for resorting and verification of identifications and counts, using the same QA/QC protocols described for the samples that are processed at Versar's laboratory. Organism identifications are performed by qualified experts and a taxonomic voucher collection is maintained. Versar closely monitors the QA/QC benthic sample protocols of its subcontractors, which are similar to Versar's.

6.0 DATA MANAGEMENT

Versar's data management procedures ensure that data meet quality objectives to answer MD DNR's questions with sufficient accuracy and precision, and are compatible and comparable with data collected in previous years of the program. Data are also compatible and comparable with those of the Virginia Chesapeake Bay Benthic Monitoring Program. An overview of the process is provided in Figure 6-1. Further detail is provided in the SOPs (Attachment 1).

All data taken in the field or lab are recorded on standard data forms designed for the project. These include field data sheets and laboratory data (bench) sheets (Attachment 2). All data to be entered into electronic files are recorded on laboratory data (bench) sheets, which are archived in project files after keypunching. Abundance and biomass data are entered into a Microsoft Access data base which already stores sample information (serial number, station number, collection date, etc.) and a list of taxa names from which the species are selected. Once the data have been entered, a report is run from the Access database and printed to provide keypunch verification. The printout is checked line by line against the bench sheet by the QA Manager. Any errors are noted in the bench sheet, and corrected in the data base by a different data entry operator. A new printout is then obtained and all corrections rechecked. This process is repeated until no further errors are found.

For all data, error- and range-checking (e.g., expected normal ranges of DO, temperature, or salinity) programs are run to identify entry errors. The output of these programs is reviewed and values outside the ranges listed in Table 6-1 are flagged for special attention. The QA Manager verifies data output files against original data sheets to ensure that the computer file is complete and correct. One program also checks abundance and biomass files to make sure the species listed in both files match. An electronic log is maintained of all data sets; progress with respect to project deadlines is closely monitored.

Prior to statistical analysis, data are summarized in a form that can be reviewed easily for actual values and for relative trends. The Lead Scientist reviews this output for disparate data points that suggest, for example, a possible error in recording a number or in the function of a meter, etc. This procedure is redundant with computerized range checking but ensures that erroneous data do not confound subsequent analyses; past experience has shown this redundant review to be essential.

If an electronic data file requires editing, the editing software maintains an audit trail (comments identifying corrections or modifications to the file). After a data file is edited, the data verification procedures described above are repeated. All files on the computer system are backed up daily. All programs that operate on data are thoroughly tested and documented.

Original data sheets are archived for reference. Data tapes and printouts are maintained in controlled central storage areas. At present, data are submitted annually to EPA's Chesapeake Bay Program Information Management System (CIMS) and posted in the Benthic Monitoring Program web site (www.baybenthos.versar.com). These data are stored in accordance with CIMS requirements, as shown in Attachment 3.

Table 6-1. Ranges of values accepted by error-checking computer programs

A. UNIVERSAL VARIABLES	
Variable	**Check**
Sample Collection Date	Within Cruise Period
Cruise Number	Match with Date
Fiscal Year Code	Match with Date
Station/Site Number	In List
Stratum Code	In List

B. FIELD DATA

Variable	Check
Sample Number	$\geq 1, \leq 4$
Gear Code	In List
Conversion Factor	Match with Gear Code
Serial Number	Cruise Serial Number Range
Depth	$> 0, \leq 35$ m
Bottom Depth	$\geq$ Depth
Salinity	$\geq 0, < 25$ psu
Conductivity	$\geq 0, \leq 45$ mmho
DO	$\geq 0, \leq 17$ ppm
pH	$\geq 6.0, \leq 9.5$
Temperature	$\geq 0, \leq 29.0°$ C

C. SEDIMENT DATA

Variable	Check
Sample Number	$\geq 1, \leq 4$
Sand Content	$\geq 0, \leq 100$ %
Silt-Clay Content	$\geq 0, \leq 100$ %

D. TAXONOMIC DATA

Variable	Check
Sample Number	$\geq 1, \leq 4$
Taxon Code	Valid, Found previously at stratum
0.5 mm Sieve Abundance	> 0

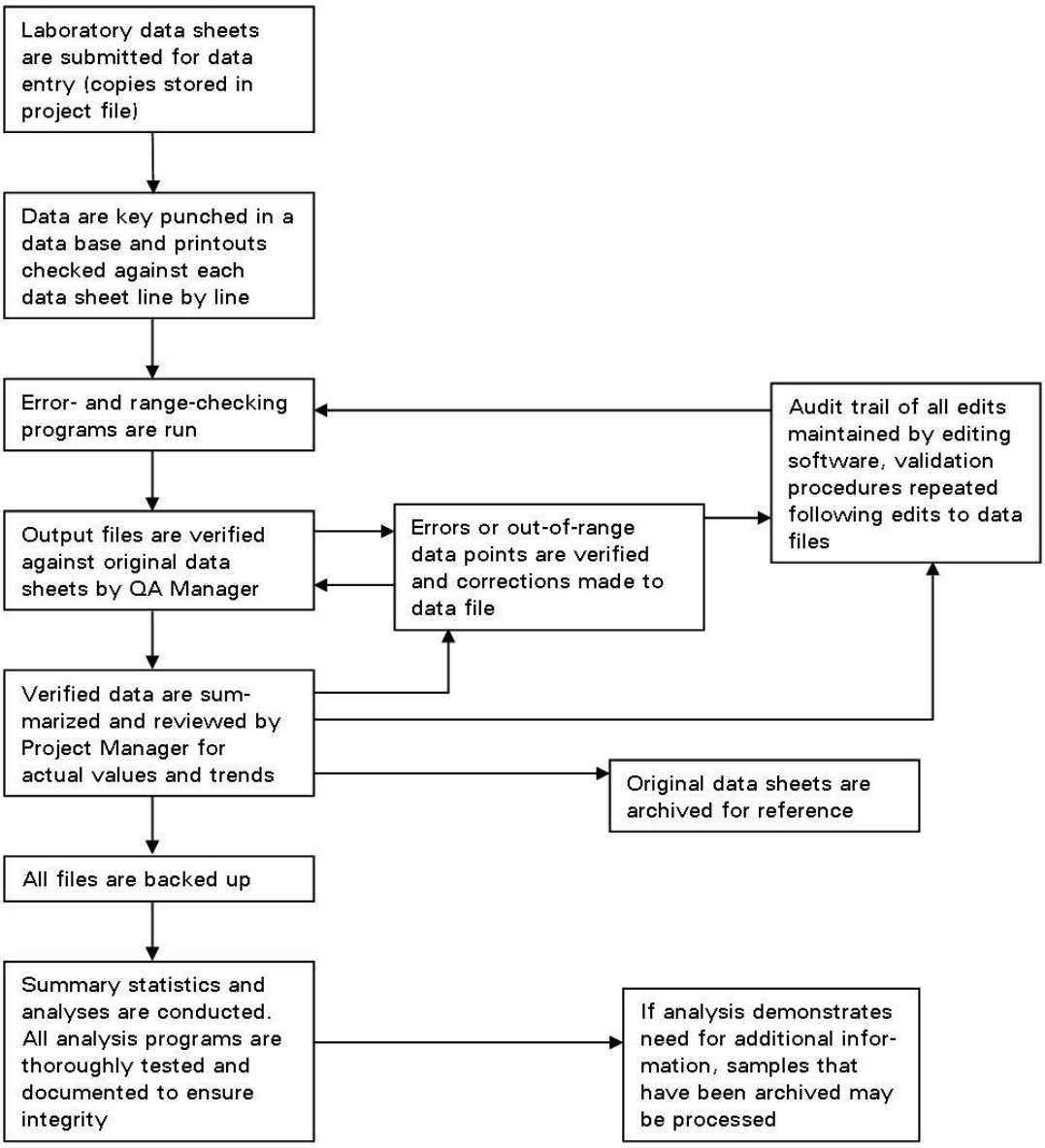

Figure 6-1. QA/QC for data processing. Further detail is provided in the SOPs (Attachment 1).

7.0 DATA ANALYSIS

Analyses for the fixed site and probability-based elements of LTB are both performed in the context of the Chesapeake Bay Program Benthic Community Restoration Goals and the Benthic Index of Biotic Integrity (B-IBI) by which goal attainment is measured. The B-IBI, the Chesapeake Bay Benthic Community Restoration Goals, and statistical analysis methods for the two LTB elements are described below.

7.1 THE B-IBI AND THE CHESAPEAKE BAY BENTHIC COMMUNITY RESTORATION GOALS

The B-IBI is a multiple-attribute index developed to identify the degree to which a benthic assemblage meets the Chesapeake Bay Program Benthic Community Restoration Goals (Ranasinghe et al. 1994, updated by Weisberg et al. 1997; Alden et al. 2002). The B-IBI provides a means for comparing relative condition of benthic invertebrate assemblages across habitat types. It also provides a validated mechanism for integrating several benthic community attributes indicative of habitat "health" into a single number that measures overall benthic community condition.

The Restoration Goals are quantitative expectations (e.g., abundance, biomass, or diversity values) based on relatively unimpacted benthic communities in Chesapeake Bay. Benthic data from several different monitoring programs were standardized to allow their integration into a single, coherent data base. From that data base a set of benthic community attributes and threshold values (the Goals) was developed to describe characteristics of benthic assemblages expected at sites having little evidence of environmental stress or disturbance. Measures used in Restoration Goal development were of five types: diversity, abundance and biomass, life history, activity beneath the sediment surface, and feeding guilds. Using these goals, benthic data from any part of the Bay could be compared to determine whether conditions at a site met, were above, or were below expectations defined for reference sites in similar habitat types. The Restoration Goals were developed for the worst-case scenario, the summer period (July 15 to September 30), when benthic communities are expected to show the greatest response to low dissolved oxygen and pollution stress.

The B-IBI is scaled from 1 to 5; sites with values of 3 or more are considered to meet the Restoration Goals. The index is calculated by scoring each of several attributes as either 5, 3, or 1 depending on whether the value of the attribute at a site approximates, deviates slightly from, or deviates strongly from values found at the best reference sites in similar habitats, and then averaging these scores across attributes. The criteria for assigning these scores are numeric and depend on habitat. The application is presently limited to summer samples; data from time periods for which the B-IBI has not yet been developed are not used for B-IBI based assessment.

Benthic community condition is classified into four levels based on the B-IBI. Values less than or equal to 2 are classified as severely degraded; values from 2 to 2.6 are classified as degraded; values greater than 2.6 but less than 3.0 are classified as marginal; and values of 3.0 or more are classified as meeting the goals. Values in the marginal category do not meet the Restoration Goals, but they differ from the goals within the range of measurement error typically recorded between replicate samples.

7.2 FIXED SITE TREND ANALYSIS

Trends in condition at the fixed sites are identified using the nonparametric technique of van Belle and Hughes (1984). This procedure is based on the Mann-Kendall statistic and consists of a sign test comparing each value with all values measured in subsequent periods. The ratio of the Mann-Kendall statistic to its variance provides a normal deviate that is tested for significance. Alpha is set to 0.1 for these tests because of the low power for trend detection for biological data. An estimate of the magnitude of each significant trend is obtained using Sen's (1968) procedure which is closely related to the Mann-Kendall test. Sen's procedure identifies the median slope among all slopes between each value and all values measured in subsequent periods.

The van Belle and Hughes procedure extends the Mann-Kendall test for use in testing for trends across multiple seasons and/or multiple strata (Gilbert 1987). Multiple-strata or multiple season tests address more global issues, such as testing for trends in the whole Potomac River, rather than a single site within the Potomac. Examining trends across multiple sites increases the power for trend detection by increasing the effective sample size. The test using combinations of sites (and/or seasons) is conducted in two parts. The first part tests for homogeneity of response across the groups to be combined. Combination is inappropriate if individual trends are significantly heterogenous (similar to the lack of validity of a two-way analysis of variance when there is a significant inter-effect interaction). In the second part, a chi-square test based on the normal deviates is used to determine the significance of the "global trend." The magnitude of the global trend is estimated by extending Sen's (1968) procedure to determine the median slope for all slopes for the multiple strata being tested (Gilbert 1987).

7.3 PROBABILITY-BASED ESTIMATION

The Maryland Bay is divided into six strata plus the deep trough (Figure 4-2, Table 4-3). To estimate the amount of area in the entire Bay that fails to meet the Chesapeake Bay Benthic Restoration Goals (P), we define for every site *i* in stratum *h* a variable y_{hi} that has a value of 1 if the benthic community meets the goals, and 0 otherwise. For each

stratum, the estimated proportion of area meeting the goals, P_h, and its variance are calculated as the mean of the y_{hi}'s and its variance, as follows:

$$p_h = \bar{y}_h = \sum_{i=1}^{n_h} \frac{y_{hi}}{n_h} \quad (1)$$

$$\text{var}(p_h) = s_h^2 = \sum_{i=1}^{n_h} \frac{(y_{hi} - \bar{y}_h)^2}{n_h - 1} \quad (2)$$

Estimates for strata are combined to achieve a statewide estimate as:

$$\hat{P}_{ps} = \bar{y}_{ps} = \sum_{h=1}^{6} W_h \bar{y}_h \quad (3)$$

where the weighting factor $W_h = A_h/A$; A_h is the total area of the *h*th stratum, and A is the combined area of all strata. The variance of (3) is estimated as:

$$\text{var}(\hat{P}_{ps}) = \text{var}(\bar{y}_{ps}) = \sum_{h=1}^{6} W_h^2 s_h^2 / n_h \quad (4)$$

For combined strata, the 95% confidence intervals are estimated as the proportion plus or minus twice the standard error. For individual strata, the exact confidence interval is determined from tables.

7.4 REPORTING

Level I Comprehensive reports are produced annually by the Long-Term Benthic Monitoring and Assessment Program. Level I reports summarize data from the latest sampling year and provide a limited examination of how conditions in the latest year differ from conditions in previous years of the study, and whether there are any changes in benthic community trends. Reports include Introduction, Methods, Results, and Discussion sections plus appendices presenting the raw biological data (abundance and biomass values by species), the B-IBI data (metric and index values and scores), and the sedimentary and hydrographic data associated with each sample. Graphic and tabular displays of data are produced using a variety of software packages and ArcView. The output is automated to minimize transcription errors in the report.

Each year, after the data have been finalized, checked, and updated in the data base, Versar uploads the data to the Long-Term Benthic Monitoring and Assessment Program website (www.baybenthos.versar.com). Computer programs convert the data into specific formats required by the Chesapeake Bay Information Management System (CIMS) and export the data into comma delimited text files. These files are then used by the Chesapeake Bay Program and entered into their data base. There are seven types of text files provided. The seven text files break down the data into categories: sampling event information, sample collection information, water quality data, sediment data,

species and abundance data, biomass data, and metric and index values and scores. The structure of such files is documented in the Maryland Chesapeake Bay Benthic Monitoring Program Data Dictionary (Attachment 3), which is updated regularly.

A public web site such as the one created for the Long-Term Benthic Monitoring Program must be visually appealing and have both high technical quality and general educational value. It also has to effectively deliver the main message of how's the Bay doing and the most salient points of the current monitoring year. The web site is maintained on a state-of-the-art server protected by corporate firewall and security practices. The content of the site consists of approximately 600 files comprising 132 Mb of web pages, graphics, documents, and data. These are organized to present information on the Chesapeake Bay benthos, the design and accomplishments of the program, and results and analysis of data at three levels of detail (stratum, basin, and Bay Program segment), as well as to provide access to program documents, latest reports, and data sets. The web site also includes data and information about the Virginia Benthic Monitoring Program, which is essential to provide a comprehensive view of how's the Bay doing. The web site activities and data availability are conducted under a Memorandum of Agreement between Versar and CIMS. The results and achievements of the program are also regularly communicated at management and scientific meetings.

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ATTACHMENT 1
STANDARD OPERATING PROCEDURES MANUAL

VERSAR, INC.
ECOLOGICAL SCIENCES AND APPLICATIONS

VERSAR BENTHIC LABORATORY STANDARD OPERATING AND QUALITY CONTROL PROCEDURES

Revised 12 May 2020

TABLE OF CONTENTS

1.0 INTRODUCTION

1.1 BACKGROUND

In recent years, monitoring and assessment of benthic communities has been used often as an indicator of living resource condition. The benthic condition integrates temporally variable environmental conditions and the effects of multiple types of environmental stress. In addition, most environmental regulations and contaminant control measures are designed to protect biological resources; therefore, information about the condition of biological resources provides a direct measure of the effectiveness of management actions to improve environmental condition and ameliorate pollution stress.

Benthic macroinvertebrates have many attributes that make them reliable and sensitive indicators of habitat quality in aquatic environments (Boesch and Rosenberg 1981, Bilyard 1987). Most benthic organisms have limited mobility and cannot avoid changes in environmental condition (Gray 1979). Benthos live in bottom sediments, where exposure to contaminants and oxygen stress are most frequent. Benthic assemblages include diverse taxa representing a variety of sizes, modes of reproduction, feeding guilds, life history characteristics, and physiological tolerances to environmental conditions; therefore, they respond to and integrate natural and anthropogenic changes in environmental conditions in a variety of ways (Pearson and Rosenberg 1978; Warwick 1986; Wilson and Jeffrey 1994; Dauer 1993). Finally, benthic organisms are important secondary producers, providing key linkages between primary producers and higher trophic levels (Virnstein 1977, Holland et al. 1980, Holland et al. 1989, Baird and Ulanowicz 1989; Diaz and Schaffner 1990).

1.2 OBJECTIVES OF THIS DOCUMENT

This document describes the Standard Operating Procedures (SOP) for all aspects of benthic macroinvertebrate sample processing. The procedures ensure that data and sediment grain size produced in the laboratory is of the highest quality. This document is intended to be used by Versar Laboratory personnel as a training manual, for procedure review, and as a description of methods for proposals. Versar's Benthic Laboratory, in existence since the late 1970s, specializes in techniques for assessing benthic communities in freshwater, estuarine, and marine environments. The procedures are based on currently accepted practices in benthic ecology (Holmes and McIntyre 1984, APHA 1985, Klemm et al. 1990). In addition, many of the methods described have been developed and refined in the Versar Benthic Laboratory over more than 20 years. For example, many of the Quality Assessment/Quality Control (QA/QC) procedures were developed or refined by Versar and were subsequently adopted by EPA's Environmental Monitoring and Assessment Program (EMAP) (Valente and Strobel 1993).

1.3 ORGANIZATION OF THIS DOCUMENT

The remainder of this document is organized into sections, one for each of the activities of Versar Benthic Laboratory personnel. Sections 2 and 3 describe Versar's system for sample receipt, storage, tracking, and disposal. Sections 4 to 8 describe the procedures for washing, sorting, identifying, and enumerating benthic macroinvertebrates in samples and the quality control procedures

used to ensure consistent and high quality data. Sections 9 to 14 describe methods used to measure benthic biomass and the associated quality control procedures. Data entry, and data entry quality control procedures are described in Sections 15 and 16. Finally, Section 17 provides a list of literature cited within this document.

VERSAR BENTHIC LABORATORY
STANDARD OPERATING PROCEDURE (SOP)

2.0 SAMPLE RECEIPT

The objective of this SOP is to detail the Versar Benthic Laboratory procedures for receiving and logging sample shipments into the laboratory and the proper documentation associated with sample receipt.

1. The Sample Custodian is responsible for receiving all sample shipments to the laboratory. In the event that the Sample Custodian is not available, the Alternate Sample Custodian is responsible for sample receipt. The client is notified the same day that the sample shipments are received.

2. Upon arrival, the sample shipment is carefully inspected. The shipping container and subsequently each individual sample are checked for intact custody seals, as well as general condition. The Laboratory Manager is notified immediately if evidence of broken seals is found. The Laboratory Manager or Project Manager will notify the client within 24 hours of arrival of any problems identified that may compromise the integrity of the samples.

3. A Chain of Custody form should be included with each incoming sample shipment. Every sample shipped should appear on the Chain of Custody form. Any problems encountered during check-in, such as broken custody seals, missing samples, or broken sample bottles, will be recorded in the remarks section of the Chain of Custody form, initialed, and dated. The Laboratory Manager or Project Manager will notify the client of any discrepancies between the Chain of Custody form and the samples actually received in the shipment within 24 hours of receipt.

4. The Sample Custodian will also check for the absence or presence of air bill(s), chain of custody forms, and any other paperwork required by the client. Each item is checked for accuracy, then signed and dated.

5. Once the Chain of Custody form is checked for completeness and all necessary comments have been recorded, the form is signed and the Versar Benthic Laboratory assumes responsibility for the samples.

6. An electronic copy of all original sample identifications will be produced and submitted to the client as evidence of sample receipt within 24 hours of receipt of a shipment. This file, referred to as the Versar Sample Tracking Log, is used to track and document all sample processing procedures. The file also contains information on sample location within Versar or if sent to subcontractors or taxonomic experts.

7. Each individual sample is assigned a unique Versar sample tracking number. The Sample Custodian consults the Sample Number Assignment Notebook and signs out the appropriate number of labels for the batch of samples. This unique Versar

number is written on the label of each sample bottle. This Versar identification number and its corresponding field sample number originally assigned by the client is recorded in the electronic Sample Tracking Log.

8. Information that is entered into the Sample Tracking Log after sample receipt includes (if available):

   a. Field sample number

   b. Replicate

   c. Versar tracking number

   d. Sampling date

   e. Number of sample jars

   f. Condition of shipping container and sample bottles

   g. Status of custody seals on shipping container and/or sample bottles (present, absent, condition - intact, broken)

   h. Status of documentation (present, absent, complete): air bills, chain of custody records, other client paperwork

   i. Discrepancies noted/actions taken to resolve the problem

   j. Location of samples (i.e., Versar storage, subcontractor, taxonomic expert).

9. Upon completion of all check-in procedures and appropriate documents, the samples are moved to the sample storage room until processing begins.

VERSAR BENTHIC LABORATORY
STANDARD OPERATING PROCEDURE (SOP)

3.0 SAMPLE STORAGE, TRACKING, AND DISPOSAL

The objective of this SOP is to document the proper storage, tracking, and disposal procedures for laboratory samples. In the event of litigation, it is necessary to have accurate records which can be used to trace the possession and handling of samples from the time of sample receipt to the time the sample is discarded.

1. Samples are removed from the sample storage room only for the purpose of laboratory analysis. To assure proper chain of custody procedures, samples are signed out on the electronic Sample Tracking Log upon removal from the sample storage room.

2. The date and initials of the technician are entered into the electronic Sample Tracking Log upon completion of any of the following sample processing procedures: sample sorting (SOP 5.0), identification (SOP 6.0), mounting (SOP 7.0), biomass (SOP 9.0 to 12.0), and QA/QC (SOP 8.0, 13.0).

3. If an entire sample or portion of a sample is sent to a subcontractor or expert, an entry is made in the Sample Tracking Log.

4. Upon approval of the client, the Laboratory Manager, and the Project Manager, and once the final data are sent to the client, the sample debris will be discarded unless the contract specifies otherwise. Disposal of the sample debris will be recorded in the Sample Tracking Log.

5. If sample specimens are not biomassed or shipped to the client, Versar will store all sample vials for no more than 1 year after submittal of the data unless the contract specifies otherwise.

VERSAR BENTHIC LABORATORY
STANDARD OPERATING PROCEDURE (SOP)

4.0 SAMPLE SIEVING

The following laboratory procedures are based upon currently accepted practices in benthic ecology (Holmes and McIntyre 1984, APHA 1985). These procedures have been adopted by the U.S. Environmental Protection Agency for use in the estuarine portion of the Environmental Monitoring and Assessment Program for the assessment of the macrobenthic community (Valente and Strobel 1993).

Benthic samples are sieved in the laboratory to ensure each sample is consistently and completely processed. Sieving in the laboratory removes the field preservative and fine sedimentary particles from the samples. Most samples are sieved using a 0.5 mm sieve; however, other sieve sizes may be used depending on the study requirements. Size fractionation of macrofauna may be required of certain studies or fractionation may facilitate sorting. In these cases, a nest of differing size sieves is used to fractionate the fauna and only the fine material that washes through the smallest size sieve is discarded. This SOP is based on the assumption that benthic samples are preserved upon field collection. The laboratory will continue to store the sample in the preservative used in the field. General procedures for sample sieving are as follows:

1. Throughout laboratory processing, all samples will be tracked by the field sample number and Versar tracking number (according to SOP 3.0). Label all samples or fraction of samples with these numbers.

2. Clean and backwash thoroughly all sieves and containers before and after sample processing. Spray the front and back of all sieves, and the interiors of all containers with water at a pressure sufficient to remove any adhered particles. Removing small stones embedded in the sieve mesh may require gentle scrubbing with a nylon bristle brush. All items should be clean prior to this process; however, this step prevents cross- contamination in the event some particles were missed.

3. Always wear a lab coat and rubber gloves when sieving samples. Under a fume hood, pour the sample through the sieve. Save the filtrate in a properly labeled container to re- preserve the sample residue once sorting is complete.

4. Place the sieve into a plastic wash basin in the sink to reduce the risk of losing any sample down the drain. Using tap water, rinse any portion of the sample remaining in the field jar into the sieve, making sure that none remains in the jar. Any time a sample is transferred from one container to another, the receiving container should be washed thoroughly. This eliminates the possibility of sample loss.

5. Fill the basin with water and agitate to wash fine material through the sieve. This procedure minimizes mechanical damage to fragile fauna. A gentle spray of water may also be used to wash material through the sieve, but direct, heavy jets of water may not be used. Periodically remove the sieve from the wash basin and replace the water with clean water.

6. When agitation ceases to sieve substantial amounts of fine particles from the sample, transfer the material from each sieve into a labeled jar in preparation for sorting. Use a gentle spray of water from a spouted water bottle.

7. Examine each sieve after rinsing to ensure that all organisms have been removed and to minimize cross contamination with the next sample.

8. Back wash all sieves with a heavy, direct spray of water and allow to dry before the next use.

VERSAR BENTHIC LABORATORY
STANDARD OPERATING PROCEDURE (SOP)

5.0 SAMPLE SORTING

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

Typically the fauna of interest for most macrobenthic studies are operationally defined as those organisms retained by a 0.5 mm (500 µm) mesh sieve. All fauna retained on the 0.5 mm sieve will be identified, enumerated, and included as macrofauna, excluding those groups more commonly regarded as meiofauna (e.g., ostracods and harpacticoid copepods). No upper size limit for macrofauna is used by the laboratory.

Consistent high quality in the sample sorting process is assured by several measures: only qualified technicians with training will sort samples; the sample sorting protocol is documented and uniformly applied to all samples; and all sorting is closely and continuously monitored by supervisory staff. In combination, the application of training, supervision, and controlled laboratory procedures ensures that all samples are processed correctly, and that resulting data are not invalidated by contamination, loss of vials, or incomplete removal of organisms from the sample. Additional quality control procedures for sample sorting are given in SOP 8.0.

The following laboratory procedures are based upon currently accepted practices in benthic ecology (Holmes and McIntyre 1984, APHA 1985). Most of these procedures have been adopted by the U.S. Environmental Protection Agency for use in the estuarine portion of the Environmental Monitoring and Assessment Program for the assessment of the macrobenthic community (Valente and Strobel 1993).

Procedures for sample sorting are as follows:

1. Remove all macrofauna alive at the time of collection from organic debris and sediment particles remaining after sieving. Sort all organisms, including all body fragments and, unidentifiable material.

2. Sorting commences by pouring material from the larger fractions (i.e., 2.0 or 4.75 mm) into gridded white enamel or plastic trays. Transfer finer material to a gridded petri dish. Evenly distribute enough sample to just cover the tray or petri dish. Add enough water to just cover the sample; water level must be low enough to prevent sloshing back and forth as the dish is moved.

3. Samples are normally sorted using a stereo microscope. For those samples in which a series of sieves was used, magnifying (10x power) fluorescent lights may be used for the larger sieve size particles (i.e., 2.0 and 4.75 mm).

4. As the tray or petri dish is systematically searched, remove all organisms from the dish and place them into alcohol-filled vials. Maintain separate vials for each major taxonomic group (typically polychaetes, oligochaetes, bivalves, insects, crustaceans, etc.).

5. Insert a small label with the proper sample identification information. This information should included at least the station and replicate numbers, Versar tracking number, collection date (if available), and the taxonomic category. Bind securely together all vials with rubber bands. Fasten a label (typically an original label from the sample) to the bundle with a rubber band if available.

6. Record on the Versar Sample Tracking Log (SOP 3.0) the number of vials for each sample, the date completed, and estimate of time spent. Also record the Versar tracking number on a QA/QC batch listing sheet located in the Versar Laboratory QA/QC Log Book. Inform the Laboratory Manager when you have sorted 10 samples so that a sorting QC can be done in a timely manner (SOP 8.0).

7. Transfer the sample debris (that remaining after sorting) from the petri dish to the original sample jar, preserve in the study preservative, label, and for each technician, save in batches of 10. A log will be kept of all samples archived. Ten percent of each batch will be resorted as a quality control check on each sorter's efficiency (SOP 8.0).

VERSAR BENTHIC LABORATORY
STANDARD OPERATING PROCEDURE (SOP)

6.0 IDENTIFICATION AND ENUMERATION

The identification of biological specimens requires specialized taxonomic training, experience, and a familiarity with current taxonomic literature. The validity of taxonomic identifications affects the quality of subsequent population and community analyses, as well as the comparability of the research to other studies. Therefore, only qualified and experienced technicians perform identifications. In some instances, rare or uncommon organisms are sent to expert taxonomists at other laboratories for assistance with identification. Versar has a good working relationship with outside taxonomic experts and utilizes their expertise when necessary. Additional quality control procedures for species identification and enumeration are given in SOP 8.0.

The objective of taxa identification and enumeration is to accurately identify all organisms found in a sample to the lowest possible taxonomic category, consistent with study objectives, and to accurately count the number of organisms in each taxonomic category. In most cases, specimens are identified to the species level (including oligochaetes and chironomids).

The following laboratory procedures are based upon currently accepted practices in benthic ecology (Holmes and McIntyre 1984, APHA 1985, Klemm et al. 1990). These procedures have been adopted by the U.S. Environmental Protection Agency for use in the estuarine portion of the Environmental Monitoring and Assessment Program for the assessment of the macrobenthic community (Valente and Strobel 1993).

General procedures for taxa identification and enumeration are as follows:

1. Sample processing for identification and enumeration commences by retrieving the bundle of vials for a particular sample produced from SOP 5.0. At that time, a species identification data sheet is started. Check the sample number on the vials with that recorded in the Versar Sample Tracking Log (SOP 2.0) and confirm that the number of vials in the bundle is the same as that listed in the log.

2. Rinse the contents of each vial into a petri dish and identify, count, and remove the specimens from the petri dish one at a time. Place the identified organisms into vials with the proper labels. Usually taxa will be placed into higher taxonomic categories as in the sorting procedure (SOP 5.0), but separate taxon vials can be produced if required by the client.

3. Record the identification and count of each taxon on the laboratory data sheet. The inside label for each taxon should include at least the station and replicate numbers, Versar Sample Tracking number, collection date (if available), and the taxonomic category.

4. All identifications are done using a high-quality dissecting microscope with

sufficient magnification for clear resolution of morphological details. A microscope with 5 to 50x power is usually sufficient. On occasion, a compound microscope capable of higher magnification may be required and is available for use.

5. All taxonomic identifications are based on current literature and the use of standard taxonomic keys such as those listed in Klemm et al. 1990. Use laboratory reference specimens to verify identifications whenever needed.

6. The number of individuals counted for each taxon must reflect the number of organisms alive at the time of sampling. Therefore, when organism fragments are recovered, counts are based upon only the number of heads found. Body fragments should be placed with the appropriate taxonomic group. If only posterior fragments are present (no heads), count these as one individual unless a greater number of individuals can be positively identified, in which case, record that count and note on the laboratory data sheet that counts included posterior ends. Count only those fragments which can be identified as constituting a unique organism.

7. When completing taxonomic identifications, some specimens cannot be completely identified to the species level, particularly if they are immature/juveniles or in poor shape. In these instances, the taxonomist will identify the specimen to the lowest practical taxonomic level, and will record on the data sheet, when it is the opinion of the taxonomist, that such a specimen should not be considered a separate taxon when tallying the total number of taxa. A separate column for such a designation is on all Versar laboratory species sheet for entry into the database.

8. Specimens which are difficult to identify should be set aside in vials, and preserved for further study. Proper identification of some specimens may require the expertise of more experienced technicians in the same laboratory. Other specimens may require further laboratory processing (oligochaetes and chironomids will need to be mounted on microscope slides, for example) before species determination can be made. Still other specimens may need to be sent to outside experts to complete species identifications. The species identification data sheet and the Versar Sample Tracking Log are used to track the location of all specimens for a particular sample (see SOP 2.0).

9. A voucher reference collection of each taxa identified by the Versar Benthic Laboratory is maintained in the laboratory. This collection is used to help train new taxonomists, verify identifications, and help resolve future taxonomic problems, should they occur.

10. A reference collection of each taxa identified for a specific project will be generated if required by the client. Each taxon vial is adequately labeled as in number 3 above. The number of specimens removed from the sample for the reference collection is recorded on the laboratory data sheet for entry into the electronic data base.

VERSAR BENTHIC LABORATORY
STANDARD OPERATING PROCEDURE (SOP)

7.0 FRESHWATER SAMPLE SPLITS AND IDENTIFICATION OF OLIGOCHAETES AND CHIRONOMIDS

In general, all specimens are identified and enumerated from visual inspection using a stereo microscope. However, oligochaetes and chironomids require special handling to optimize taxonomic identification. Specimens of both groups need to be mounted on slides for proper taxonomic identification. Sample processing of oligochaetes and chironomids will proceed in the following manner for each group unless otherwise stipulated by the client in the laboratory contract.

The following laboratory procedures are based upon currently accepted practices in benthic ecology and have been modified and refined within the Versar Benthic Laboratory. These procedures have been adopted and modified by the U.S. Environmental Protection Agency for use in the estuarine portion of the Environmental Monitoring and Assessment Program for the assessment of the macrobenthic community (Valente and Strobel 1993).

1. For each sample and for each group (oligochaetes and chironomids), if less than 20 individuals are found in a sample, then all individuals will be permanently mounted and identified to the lowest possible taxonomic level. Record on the laboratory data sheet that 100% of the specimens were slide mounted.

2. If the number of specimens is greater than 20, split the samples in the following manner:

    a. Spread the specimens in a gridded tray as evenly as possible. Randomly select grids until at least 35 specimens are mounted. Mount any specimens remaining in the last selected grid. Thus, the total number of mounted specimens in these samples will usually be greater than 35.

    b. Save in a labeled vial the specimens in the remaining grids. Include on the label the split value which equals the percentage of specimens that were slide mounted.

    c. Record the percentage of specimens that were slide mounted on the laboratory data sheet.

4. Three or more oligochaete specimens can be placed under a microscope cover slip at one time. Be sure that specimens are similar in size if mounting several at one time. In addition, be sure that all heads are facing in the same direction to facilitate identification.

5. Only one chironomid per cover slip should be mounted. The head should be removed from the body (unless the specimen is very small) to facilitate the correct positioning of

the head capsule for identification.

6. A permanent mounting medium such as CMC-10 should be used.

7. Allow the slides to dry over night and add more mounting medium should air bubbles form. The slides should be allowed to dry for several days before specimen identification.

8. Adjust on the laboratory data sheet the total taxonomic counts of each taxon by the proportion of total number of oligochaetes or chironomids mounted in the sample.

VERSAR BENTHIC LABORATORY
STANDARD OPERATING PROCEDURE (SOP)

8.0 QUALITY CONTROL FOR SAMPLE SORTING, IDENTIFICATION, AND ENUMERATION

Various quality control procedures are implemented to ensure the consistent production of high- quality data. The procedures in this SOP were developed by Versar Benthic Laboratory personnel with the assistance of a biological statistician to provide effective and continuous monitoring of laboratory personnel. Many of these procedures have been adopted by the U.S. Environmental Protection Agency for use in the estuarine portion of the Environmental Monitoring and Assessment Program for the assessment of the macrobenthic community (Valente and Strobel 1993).

Typically, the minimum acceptable laboratory efficiency is 90% as defined below. However, based upon the experience of the Versar Benthic Laboratory, efficiency is expected to be greater than 95%. If laboratory efficiency required by a specific client is greater or less than 90%, then the required efficiency will be specified in the laboratory contract and the following procedures amended as necessary to achieve the desired efficiency.

8.1 SORTING

1. A minimum of 10% of all samples sorted by each technician is resorted to monitor technician performance and provide feedback necessary to maintain acceptable standards. Resorts are conducted on a regular basis on batches of 10 samples and all results documented in the QA/QC log book for the laboratory.

2. For each technician, for each batch of ten, one sample is randomly selected for resorting from a sample batch.

3. Retrieve and log into the QA/QC log book the selected archived sample residue.

4. Resort the residue using the sorting procedures given in SOP 4.0 and 5.0.

5. Calculate sorting error (%) using the following formula:

$$\frac{\text{\# organisms found in QC inspection}}{\text{\# of organisms originally sorted + additional \# found in resort}} \times 100$$

6. The results of sample resorts may require that remedial actions be taken. If the sorting error is less than 5%, then no action is required. If the sorting error is between 5 and 10%, the technician is retrained and problem areas identified. Laboratory personnel and supervisors must be particularly sensitive to systematic errors (e.g., consistent failure to represent specific taxonomic groups) which may suggest the need for further training. If the sorting error is greater than 10%, all of the samples in that batch will be resorted by the technician. The performance of the inefficient technician must be continuously

monitored until efficiency is improved to the desired level.

7. If the sorting error is greater than 10%, add the organisms found in the resort to the original data sheet and the respective vials.

8. Place all specimens found during the resorting of the failed batch into the respective vials for each sample.

9. Record all QA/QC results on the QA/QC data sheet.

10. After resorting, another QC is performed and Steps 3 through 9 are repeated until the batch passes the 10% error criterion.

11. After resorting, and if quality control criteria are met, sample residues may be discarded unless otherwise stipulated by the client.

8.2 SPECIES IDENTIFICATION AND ENUMERATION

1. Only senior taxonomists are qualified to complete identification quality control checks. This control check establishes the level of accuracy with which identification and counts are performed and offers feedback to taxonomists in the laboratory so that a high standard of accuracy is maintained.

2. Approximately 10% of each sample batch is checked. A sample batch is 10 samples and ideally is made of samples from similar habitat type (i.e., all tidal freshwater samples). Conduct these rechecks in a timely manner so that subsequent processing steps and data entry may proceed.

3. Retrieve the specimen vials and microscope slides (if oligochaetes and chironomids are present in the sample) of a randomly selected sample from a sample batch along with the original species identification sheet.

4. Identify and enumerate the specimens in each sample using the procedures given in SOP 6.0, and record the taxonomic name and count on a re-identification QA/QC data sheet.

5. After each taxon is re-identified and re-counted, double check discrepancies with the original data sheet to ensure accurate final results.

6. Following re-identification, return the specimens to the original vials.

7. When the entire sample has been re-identified, calculate the total number of errors. The total number of errors is based upon the number of misidentifications and miscounts. Numerically, the percent identification error is represented in the following manner:

$$\frac{\text{Total \# of identification/enumeration errors*}}{\text{Total \# of organisms in the sample}} \times 100$$

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

1. Counting errors (for example, counting 11 chironomids as 10)

2. Identification errors (for example, identifying a *Limnodrilus hoffmeisteri* specimen as *Limnodrilus udekemianus*, where both are present)

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

8. If the QA/QC results are greater than 10%, the entire sample batch is re-identified and counted. Changes in counts or identifications based on the QA/QC procedures are recorded on the original laboratory data sheet.

9. If the identification error is below 10%, the original technician is advised and any errors in species identifications are reviewed. Record all changes in species identification on the original data sheet and enter these changes into the database. However, do not correct the numerical count for each taxonomic group unless the overall accuracy for the sample is below 90%.

10. Record the results from all QC rechecks of species identification and enumeration on the re- identification data sheet.

11. Initial and date all corrections made to the original laboratory data sheets.

VERSAR BENTHIC LABORATORY
STANDARD OPERATING PROCEDURE (SOP)

9.0 MACROFAUNAL DRY-WEIGHT BIOMASS DETERMINATION

Biomass is individually determined for the most dominant macrofaunal taxa or group of taxa present in each study. Dominant taxa or groups of taxa are selected based on the taxa dominance of the study samples after laboratory identification, unless preassigned categories are required by the client. Grouping of taxa into categories is based on ecological or taxonomic relevance.

The following dry-weight biomass laboratory procedures are based upon currently accepted practices in benthic ecology and are applicable for all organisms without calcium carbonate shells or exoskeletons. The procedures for shell dissolution are found in SOP 11.0. Many of these procedures have been adopted by the Environmental Protection Agency for use in the estuarine portion of the Environmental Monitoring and Assessment Program for the assessment of the macrobenthic community (Valente and Strobel 1993).

1. The measurement of biomass for each taxa category commences with the collection of the species identification data sheet and the taxon specimen vials for an individual sample. Biomass data sheets accompany abundance sheets and note how many and which biomass vials should be present. Correct any discrepancies between data sheets and vials at this time.

2. Measure biomass using an analytical balance with an accuracy of 0.1 mg.

3. Place all organisms, other than ones with calcium carbonate shells, in ashed, aluminum pans. The pans are made by forming squares of aluminum foil into crucible-shapes using an appropriately sized crucible as a form. Ash the pans as in SOP 10.0, step 2.

4. Prior to weighing pans and crucibles, zero the balance and using a standard weight, test its calibration. Record all calibrations in the laboratory calibration notebook. See SOP 14.0 for further details about balance use.

5. Place the organisms in pre-weighed, aluminum weighing pans and then in numbered crucibles. Select an appropriately sized pan for each taxonomic category according to the amount of material to be processed. Record the crucible number on the biomass data sheet along with the taxonomic group to be measured.

6. Take care to check that all organisms are rinsed from the vials into the weighing pan.

7. Place the weighing pans and crucibles in carrying trays and dry in an oven at 60°C for at least 24 hours. Record the date and time the samples are placed in the oven next to the batch number on the tray.

8. Typically, 24 to 48 hours is sufficient for the dry-weight of benthic samples to stabilize. However, some samples may take longer to dry. As a check, weigh all

samples after 24 hours, record the weight on the biomass data sheets, and return the samples to the drying oven. Reweigh the samples after an additional 24 hours. If the second sample weight differs from the first by more than 10%, return the sample to the drying oven for an additional 24 hours. Repeat this cycle until a stable dry-weight measurement is obtained. Record all weights on the biomass data sheet.

9. Group the pans and crucibles to be weighed in batches. One carrying tray constitutes one batch, and batches are numbered sequentially from the first day biomass processing begins, and alphabetically from the first batch each day. For example, on day one if four batches are started, they are numbered 1A, 1B, 1C, and 1D. On day two, if four more batches are started, they are numbered 2A, 2B, 2C, and 2D. Record the batch number on biomass data sheets and on each batch. To determine the overall accuracy of the weighing procedure and help detect errors due to the contamination of biomass samples, each batch contains weighing blanks. Blanks are pans and crucibles which have been treated as biomass samples but to which no fauna have been added. Approximately 5 to 10% of the pans and crucibles in a batch are blanks.

10. Samples must be cooled to room temperature before weighing. Store dried samples awaiting measurement in a desiccator to avoid absorbing moisture from the atmosphere while cooling.

11. After weighing a batch, have another technician reweigh approximately 10% of all pans and crucibles as a quality control check of biomass measurements.

12. Laboratory biomass data sheets contain a record of all weights. However, only the final dry- weight of each taxonomic category will be forwarded to the client, unless otherwise stipulated.

13. After the completion of all weighing procedures, blanks should vary by no more than 0.3 mg. If greater variations are found, the balance and the procedures used by the technician in its operation will be checked and the balance repaired, or the technician retrained, as necessary.

VERSAR BENTHIC LABORATORY
STANDARD OPERATING PROCEDURE (SOP)

10.0 MACROFAUNAL ASH-FREE DRY WEIGHT BIOMASS DETERMINATION

Biomass is individually determined for the most dominant macrofaunal taxa or group of taxa present in each study. Dominant taxa or groups of taxa is selected based on the taxa dominance of the study samples after laboratory identification, unless preassigned categories are required by the client. Grouping of taxa into categories is based on ecological or taxonomic relevance.

The following ash-free dry weight procedures are based upon currently accepted practices in benthic ecology (Holmes and McIntyre 1984).

1. To determine ash-free dry weights, first complete the dry weight procedures (SOP 9.0), then continue with the following procedures.

2. Fire all weighing pans and crucibles used in this procedure in a muffle furnace for 3 hours at 500-550 °C before each use. After firing, use gloves or forceps to prevent touching which can affect pan weights.

3. After the final dry weight is obtained, close the tops of the aluminum pans, and fold over. This prevents ash from escaping. Place weighing pans and crucibles in a muffle furnace, cover all crucibles with lids and ash for 3 hours at 500-550°C. Muffle furnace warm-up and cool-down time (approximately 1 hour each) are not included in the 3 hour time period.

4. After ashing, transfer the crucibles to the drying oven for at least one hour. Transfer the samples from the drying oven to a desiccator, allow to cool to room temperature, then weigh with the analytical balance.

5. Weigh each ashed pan or crucible and record all weights on the laboratory biomass sheets. However, only the ash-free, dry-weight of each taxa group will be forwarded to the client, unless otherwise stipulated.

6. After the completion of all weighing procedures for each batch, blanks should vary by no more than 0.3 mg. If greater variations are found, the balance and the procedures used by the technician in its operation should be checked and the balance repaired, or the technician retrained, as necessary.

VERSAR BENTHIC LABORATORY
STANDARD OPERATING PROCEDURE (SOP)

11.0 MACROFAUNAL BIOMASS DETERMINATION FOR HARD-BODIED ORGANISMS

In some instances, clients may require the removal of inorganic, structural body parts that make up the majority of the biomass of certain hard-bodied organisms (e.g., the shell of bivalves and gastropods). In these instances, removal of the shell prior to measuring dry-weight is necessary. Acidification removes the calcium carbonate present, leaving behind organic carbon. Most of the following procedures have been adopted by the U.S. Environmental Protection Agency for use in the estuarine portion of the Environmental Monitoring and Assessment Program for the assessment of the macrobenthic community (Valente and Strobel 1993). *Note that when determining ash-free dry weight, removal of shells by acidification is not necessary, as shells remain in the ash after combustion. Shells are crushed open and placed in pre-weighed crucibles.*

1. Place hard bodied organisms in pre-weighed, numbered, porcelain crucibles. Select an appropriately sized crucible for each taxonomic category, according to the amount of material to be processed. Record the crucible number on the biomass data sheet along with the taxonomic group to be measured.

2. Take care to check that all organisms are rinsed from the vials into the crucible.

3. Shuck large bivalves (length >2cm) and process only the organic tissue since acidification of large shells takes too much time and uses an excessive amount of acid.

4. Acidify crucibles in a fume hood using 10% HCl. Add 10% acid as needed to complete the dissolution of shell material. Acidification will continue until there are no visible traces of shell material.

5. After all visible traces of shell material have disappeared, rinse the crucible with distilled water to remove the HCl byproducts.

6. For subsequent steps for biomass determination of hard-bodied organisms proceed with Steps 5 through 12 of SOP 9.0 or Steps 1 through 6 of SOP 10.0.

VERSAR BENTHIC LABORATORY
STANDARD OPERATING PROCEDURE (SOP)

12.0 MACROFAUNAL WET-WEIGHT BIOMASS DETERMINATION

Biomass is individually determined for the most dominant macrofaunal taxa or group of taxa present in each study. Dominant taxa or groups of taxa are selected based on the taxa dominance of the study samples after laboratory identification, unless preassigned categories are required by the client. Grouping of taxa into categories is based on ecological or taxonomic relevance.

The following wet-weight biomass laboratory procedures are based upon currently accepted practices in benthic ecology (Holmes and McIntyre 1984).

1. The measurement of biomass for each taxa category commences with the collection of the species identification data sheet and taxon storage vials for an individual sample. Biomass data sheets accompany abundance sheets and note how many and which biomass vials should be present. Correct any discrepancies between data sheets and vials at this time.

2. Measure biomass using an analytical balance with an accuracy of 0.1 mg.

3. Prior to weighing pans and crucibles, zero the balance and test its calibration with a standard weight.

4. Soak the organisms in distilled or deionized water for 30 minutes.

5. Blot dry all individuals of a taxa category to remove excess liquid, place in a pre-weighed pan or crucible and weigh on an analytical balance. Take care to check that all organisms are rinsed from the sample vials before weighing.

6. Record weights of each weighing pan and wet-weights of individual taxa categories on laboratory biomass data sheets. However, only the wet-weight biomass of each taxonomic category will be forwarded to the client, unless otherwise stipulated.

7. A single technician is responsible for all wet-weight biomass processing completed for a client. This avoids the introduction of technician error, especially during the crucial step of blotting the excess liquid from the organisms.

VERSAR BENTHIC LABORATORY
STANDARD OPERATING PROCEDURE (SOP)

13.0 QUALITY CONTROL PROCEDURES FOR BENTHIC BIOMASS DETERMINATIONS

To ensure that biomass measurements are standardized, benthic laboratory personnel with assistance from a biological statistician developed the following quality control procedures. Many of these procedures have been adopted by the U.S. EPA for use in the estuarine portion of the Environmental Monitoring and Assessment Program for the assessment of the macrobenthic community (Valente and Strobel 1993).

1. Whenever possible, a single technician is responsible for all biomass processing. This is particularly important for wet-weight biomass determinations in order to avoid the introduction of technician error.

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

3. Samples for weighing are randomly selected from a sample batch.

4. Selected samples are reweighed and the results compared against the final weight recorded on the biomass data sheet.

5. Weighing efficiency is calculated using the following formula:

$$\frac{\left|\text{(Original Weight - Reweighed Final Weight)}\right|}{\text{Reweighed Final Weight}} \times 100$$

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

7. Corrections to the original data sheet are made in only those cases where weighing efficiency is less than 90%.

8. The results of all QC reweighings are recorded in the QA/QC log book.

9. Additional quality control procedures (i.e., calibration of balances and use of blanks) are provided in SOP 9.0.

VERSAR BENTHIC LABORATORY
STANDARD OPERATING PROCEDURE (SOP)

14.0 USE OF THE ANALYTICAL BALANCE

Proper use and care of the balance ensures accurate measurements. The procedures for general use are presented below; detailed descriptions are found in the manufacturer's instruction manual maintained in the lab. The lab manager may also be consulted for clarification of these procedures and should be consulted if the digital display on the balance reads anything other than the values described below. The balance is also checked periodically by a qualified service technician.

General Care:

1. Clean the balance before and after every day's use.

2. Always close all balance doors before weighing.

3. If any spills occur, clean them immediately.

4. Calibrate and verify calibration before each daily use.

The following procedures describe the daily care and use of the balance:

1. To clean the balance, brush gently with the soft-bristle brush maintained in the lab for this purpose. If necessary, as determined by visual inspection, also clean the pan with an alconox solution and then dry it completely. Wear rubber gloves during cleaning to protect the pan from contact with body oils.

2. Turn the balance on by briefly pressing the control bar. After a few seconds the digital display should read 0.0000. If a different number is displayed (e.g., 0.0001), check to make sure the doors are closed and the balance is clean, then tare the balance by pressing the control bar again.

3. Check the calibration using the standard weights maintained in the lab. Only the plastic forceps in the standard weight box may be used to handle these weights. The standard weight used should reflect the lower and upper ranges of the items to be weighed. For example, if the items range from 0.0150g to 9.5000g, confirm calibration with the 0.0100 and 10.0000g standards.

    a. Place a weight on the pan and close the windows.

    b. When the display stabilizes, record the weight, the standard weight used, and the date in the calibration book. Repeat with another weight.

5. Have the balance serviced by a balance service technician on an annual basis to ensure balance accuracy and maintain balance integrity.

VERSAR BENTHIC LABORATORY
STANDARD OPERATING PROCEDURE (SOP)

15.0 DATA ENTRY & CORRECTION ON DATA SHEETS

The purpose of this SOP is to outline the proper procedures for the recording and correcting of data and observations on laboratory data sheets. Entries must be made according to the following guidelines:

1. All entries are made in black or blue ink.

2. Entries must be neat and legible, especially numerical data.

3. Errors are corrected by drawing a single line through the error and entering the correct information, initialing, and dating the correction. No original information is erased, marked out, or otherwise made illegible.

4. All unused portions of documents are "z'd" out.

5. Corrections made on the data sheets due to QA/QC procedures are made as in 3 above with a single line through the error. The taxonomist making the correction then initials the change and indicates on the data sheet that it was a QC correction.

VERSAR BENTHIC LABORATORY
STANDARD OPERATING PROCEDURE (SOP)

16.0 DATA ENTRY INTO AN ELECTRONIC FILE

The purpose of this SOP is to ensure accurate data entry from the laboratory data sheets into an electronic file. Accurate data entry is crucial to developing reliable data sets to be used for analyses. The following procedures have been developed and modified by Versar based on decades of experience in creating and maintaining large volumes of benthic data.

Data entry begins with the design of appropriate laboratory data sheets that are convenient for the taxonomist and which can easily and accurately be entered into a permanent computer data set. The Laboratory Manager and the Data Base Manager work closely together before sample processing begins to create laboratory data sheets using all available information about the geographical sampling location, sediment habitat, and water quality information for the project.

Versar has adopted a species coding system as the method for entering taxon information into the Versar data base. A master list for these species codes are maintained and updated by the Data Base Manager.

Data entry into an electronic data base is made according to the following guidelines:

1. Before data entry begins the Laboratory Manager and the Data Base Manager set up a master data entry file.

2. All data sheets are keypunched into a file by a technician. The Laboratory Manager reconciles any discrepancies when a taxonomic code does not match an existing entry in the Versar master list.

3. Taxonomic names and count entries are visually checked against the original entry in each data sheet.

4. Corrections are noted on the data sheets and each correction is entered into the electronic data base.

5. Numbers 3 and 4 above are repeated until no differences between the laboratory data sheets and the electronic data base are detected.

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ATTACHMENT 2
DATA FORMS

DEPLOYMENT RECORD

File Name: _____ Site: _____
Date Deployed: _____ Date Recovered: _____
Sonde ID: 00D0213AB DO Probe S/N: _____
Turbidity Probe S/N: _____ Chlorophyll S/N: _____
pH S/N: _____

CALIBRATION AND SENSOR TESTING INFORMATION

Date of Calibration: _____ Technician: _____

DO Membrane Changed? Y N NOTE: SHOULD WAIT 6 TO 8 HOURS BEFORE FINAL CALIBRATIONS

Turbidity wiper changed? Y N Wiper Parks ~180° from optical sensor? Y N

Record Battery Voltage: _____ V

Calibration Values

	Actual	Pre-Calibration	Post-Calibration
Conductivity	58.6 1.412	_____	_____
PH	4 7	_____	_____
PH	7 10	_____	_____
Turbidity	0	_____	_____
Turbidity	100	_____	_____
Chlorophyll	0	_____	_____
Chlorophyll	_____	_____	_____
ORP	_____	_____	_____
DO (_____ mmHg)	_____	_____	_____
DO CHARGE	_____	Should fall between 25 and 75	

POST CALIBRATION AND TESTING

	Actual	Pre-Calibration	Post-Calibration
Conductivity	58.6 1.412	_____	_____
PH	4 7	_____	_____
PH	7 10	_____	_____
Turbidity	0	_____	_____
Turbidity	100	_____	_____
Chlorophyll	0	_____	_____
Chlorophyll	_____	_____	_____
ORP	_____	_____	_____
DO (_____ mmHg)	_____	_____	_____
DO Charge	_____		

Sensors Fouled? Y N If yes, which sensors? _____
Battery Voltage _____ V

NOTES / COMMENTS (Continue on back if necessary):

Water Quality Sonde Calibration Sheet

Attachment 2-4

Maryland Dept. of Natural Resources
Chesapeake Bay Benthic Monitoring Program Field Data Sheet

Cruise AUG06 **Yearcode** 06/07 **Cruise #** 1 **Date** _____

Station HIS-001 **Site** 00001 **Time** _____

Latitude _____ **Longitude** _____ **Depth** _____

Depth (m)	Salinity (ppt)	ORP	Cond. (mmhos)	DO (ppm)	pH	Temp (C)

Rep	Serno	Gearcode	DOG (cm)
01	LTBA20990	BC-PH	
02	LTBA20991	BC-PH	
03	LTBA20992	BC-PH	

Comments:

Field Data Sheet

Attachment 2-5

Pg 1of 3 Versar Inc. Chain of Custody

LOT #	Sampler
Contact name	Project Name
Contact phone	Contract/P.O. Number
Invoice AdPTess	Sample Turnaround Time

LL USE LAB SER. NO:	Grab/ Composite	Station Location	Collection Date	Preservative	Number of Containers	Analyses Required/Comments
Total number of samples per page				Number of Jars		

Transferred by:	Received by:	Cooler Receipt Information (LAB USE ONLY)
Date:	Date:	
Transferred by:	Received by:	
Date:	Date:	

Chain of Custody Form

Maryland Dept. of the Natural Resources
Chesapeake Bay Benthic Monitoring Program Abundance (Mesohaline) Data Sheet

Yearcode 06/07 **Cruise Number** 1 **Serial Number** LTBA20990

Station HIS-001 **Rep** 01

Species	Skip	Code	Total
Glycera dibranchiata		316	
Glycinde solitaria		415	
Heteromastus filiformis		416	
Eteone heteropoda		411	
Laeonereis culveri		417	
Leitoscoloplos robustus		432	
Leitoscoloplos sp.		2965	
Loimia medusa		418	
Marenzelleria viridis		430	
Mediomastus ambiseta		2208	
Neanthes succinea		420	
Paraonis fulgens		1211	
Paraprionospio pinnata		427	
Pectinaria gouldii		423	
Podarkeopsis levifuscina		1119	
Polydora cornuta		426	
Scolelepis texana		1212	
Sigambra tentaculata		1120	
Spiophanes bombyx		1215	
Streblospio benedicti		433	
Tharyx sp. A (Morris)		1227	
Acteocina canaliculata		536	
Rictaxis punctostriatus		537	
Ensis directus		518	
Gemma gemma		519	
Geukensia demissa		2633	
Haminoea solitaria		541	
Ischadium recurvum		516	
Littoridinops tenuipes		1251	
Lyonsia hyalina		1029	
Macoma balthica		520	
Macoma mitchelli		521	
Mulinia lateralis		522	
Mya arenaria		523	
Mytilopsis leucophaeta		329	
Odostomia engonia		2974	
Parvilucina crenella		1217	
Rangia cuneata		526	
Sayella chesapeakea		667	
Tagelus plebeius		525	
Tellina agilis		1255	

Species	Skip	Code	Total
Tellinidae sp.		1699	
Ameroculodes spp. complex		468	
Apocorophium lacustre		635	
Balanus improvisus		1030	
Chiridotea almyra		625	
Cyathura polita		497	
Cyclaspis varians		2178	
Edotea triloba		498	
Gammarus daiberi		658	
Gammarus juveniles		967	
Lepidactylus dytiscus		465	
Leptocheirus plumulosus		466	
Leucon americanus		481	
Melita nitida		467	
Mucrogammarus mucronatus		463	
Americamysis almyra		2616	
Americamysis bigelowi		1300	
Neomysis americana		108	
Stylochus ellipticus		556	
Diadumene leucolena		456	
Edwardsia elegans		1125	
Leptosynapta tenuis		1224	
Nemertinea		233	
Carinoma tremaphorus		1240	
Micrura leidyi		551	
Phoronis architecta		963	
Saccoglossus kowalevskii		2036	
Oligochaetes		974	
Tubificoides sp.		1039	
Chironomidae larvae		787	
No Organisms Present		3100	

Page 1 of 219

Abundance Data (Bench) Sheet. This data sheet is for mesohaline sites. Similar data sheets are produced for tidal fresh, oligohaline, and polyhaline sites.

Biomass Bench Sheet

Serial Number LTBA20990 **Station** HIS-001 **Rep** 01 **Yearcode** 06/07 **Cruise #** 1

Staeq85 001 **Staeq89** HIS **Site** 00001 **Sample Typ** F **Gearcode** BC-PH

Name	NODC	Group	Crucible #	Cru+Dry(1)	Cru+Dry	Cru+Ash
Gemma gemma	5515471301					
Glycinde solitaria	5001280104					
Heteromastus filiformis	5001600201					
Lepidactylus dytiscus	6169220901					
Micrura leidyi	4303020505					
Mulinia lateralis	5515250301					
Streblospio benedicti	5001431801					

Thursday, May 24, 2007 **LTB Biomass Bench Sheet** Page 1 of 63

Biomass Data (Bench) Sheet. The biomass data sheet lists only the species for which there was abundance entered in the Abundance Data Sheet.

SILT/CLAY DATA SHEET

Project:

		Sand		Silt/Clay			
Sample ID	Wet Wgt.(g)	Cruc. #	Cruc+sand Wgt. (g)	Total Vol. (mL)	Sample Vol. (mL)	Cruc. #	Cruc+silt/clay Wgt. (g)

Silt/clay Data Sheet

Batch #

QC SAMPLE RESORT SHEET

Project_____ Station/Rep_____

Collection Date_____ Original Sorter_____

Additional Organisms Found

Taxa	Number	Taxa	Number

Total organisms originally sorted_____

Total additional organisms found _____

% error _____

Resorted by_____

QC OK'd _____

Any remedial action necessary_____

_____

_____

Comments_____

_____

_____

_____

Data Form - **QC SAMPLE REIDENTIFICATION SHEET**

Project _____ Collection Date _____

Station _____ Original Processor _____

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

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

No. of Misidentifications _____

Total No. of Errors _____

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

QC'd by _____ Date _____

Other problems with Sample:

Individual placed in wrong vial _____

Counts records on wrong line _____

Inadequate labels _____

Other _____

QC Sample Reidentification Sheet

CHESAPEAKE BAY BENTHIC MONITORING PROGRAM
SUMMER 2006 BENTHIC SAMPLE QC INFORMATION

Total number of samples = 231

SORTING PERCENT ERROR

Serial Number	# Errors	Original Count	Total Count	% Sorting Error
21201	0	25	25	0.00%
21040	0	166	166	0.00%
21022	1	29	30	3.33%
21170	0	19	19	0.00%
21245	1	29	30	3.33%
21084	1	20	21	4.76%
21072	1	91	92	1.09%
20996	0	33	33	0.00%
21009	0	18	18	0.00%
21011	1	31	32	3.13%
21142*	1	29	30	3.33%
21221	0	0	0	0.00%
21155	0	161	161	0.00%
21203	1	21	22	4.55%
21163	1	38	39	2.56%
21223	0	17	17	0.00%
21093	5	58	63	7.94%
21183	1	46	47	2.13%
21119	0	27	27	0.00%
21116	1	11	12	8.33%
21121	7	135	142	4.93%
21018	1	41	42	2.38%
21069	1	33	34	2.94%
Average error rate**				**2.38%**

Number of sorting QC's = 23

IDENTIFICATION PERCENT ERROR

Serial Number	# Errors	Total Count	% ID/Count Errors
20991	0	17	0.00%
20995	0	15	0.00%
20999	0	8	0.00%
21020	1	52	1.92%
21026	2	44	4.55%
21038	0	26	0.00%
21049	0	14	0.00%
21064	0	6	0.00%
21067	1	24	4.17%
21088	5	418	1.20%
21097	0	6	0.00%
21103	1	48	2.08%
21109	0	11	0.00%
21128	1	55	1.82%
21139	0	19	0.00%
21163	2	38	5.26%
21182	0	37	0.00%
21186	0	1	0.00%
21200	0	9	0.00%
21203	0	19	0.00%
21206	3	64	4.69%
21228	0	23	0.00%
21237	3	65	4.62%
Average error rate			**1.32%**

Number of taxonomic QC's = 23

*Batch failed initial QC. After batch was resorted another QC was performed.
** Average error rate only includes samples that passed the QC check as any batch that failed was resorted and reQC'ed.

Attachment 2-11

Attachment 2-12

ATTACHMENT 3
DATA DICTIONARY

MARYLAND CHESAPEAKE BAY PROGRAM BENTHIC MONITORING DATA DICTIONARY

(Revised: May 10, 2021)

ABSTRACT:
The state of Maryland, in cooperation with the USEPA Chesapeake Bay Program has monitored benthic species abundance and biomass in the Maryland Chesapeake Bay mainstem and tributaries since July 1984. This monitoring effort began as an extension of ongoing Power Plant monitoring studies in the state. The current program is designed to give comprehensive spatial and temporal information on benthic conditions in the Chesapeake Bay. The sampling parameters include water quality and sediment measurements, benthic infauna composition and abundance, and benthic infauna biomass. Sample collection is currently performed once a year, independently from Maryland plankton and water quality monitoring programs.

DATA FILE NAMING CONVENTION:

MDBEyy_EV.TXT	Maryland Benthic Program Sampling Event Record
MDBEyy_SMP.TXT	Maryland Benthic Program Sample Collection Record
MDBEyy_WQ.TXT	Maryland Benthic Program Water Quality Data Record
MDBEyy_SED.TXT	Maryland Benthic Program Sediment Data Record
MDBEyy_TX.TXT	Maryland Benthic Taxonomic and Abundance Data Record
MDBEyy_BM.TXT	Maryland Benthic Biomass Data Record
MDBEyy_IBI.TXT	Maryland Benthic Index of Biotic Integrity Record

Data files are provided in comma delimited ASCII format with header line.

ASSOCIATED DATA FILES:

NEWCODE.TXT	Species Code Supplement for the data reporting year
MISSDOC.TXT	Missing data for the reporting year
METHODCHANGE.TXT	Changes to methods for the reporting year

NAMES AND DESCRIPTIONS OF ASSOCIATED DATA DICTIONARY FILES:
Chesapeake Bay Water Quality Monitoring Program, Long-Term Benthic Monitoring and Assessment Component Quality Assurance Project Plan (QAPP)

Please see QAPP at http://www.baybenthos.versar.com/data.htm for detailed information on program organization and management, program objectives, program design, station location and identification procedures, field collection procedures, laboratory processing, data management and analysis, and data quality assurance and control (QA/QC) procedures.

PROJECT TITLE:
Maryland Chesapeake Bay Long-Term Benthic Monitoring and Assessment Program

CURRENT PRINCIPAL INVESTIGATORS:
Program Manager: Tom Parham, Maryland Department of Natural Resources, Tidewater Ecosystem Assessment.
Program Lead Scientist: Dr. Roberto J. Llansó, Versar, Inc.
Data Coordinator: Michael F. Lane, Old Dominion University.

CURRENT FUNDING AGENCIES:
Maryland Department of Natural Resources as match grant to U.S. Environmental Protection Agency Chesapeake Bay Program.

CURRENT QA/QC OFFICER:
Suzanne Arcuri, Versar-ERG

POINT OF CONTACT:

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   Columbia, Maryland 21045-1934
   Direct: 410-740-6052
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2. Mike Mallonee
   Water Quality Data Manager
   Interstate Commission on the Potomac River Basin
   USEPA Chesapeake Bay Program Office
   410 Severn Ave, Suite 109
   Annapolis, Maryland 21403
   Direct: 410-267-5785
   Fax: 410-267-5777
   E-Mail: mmallone@chesapeakebay.net

LOCATION OF STUDY:
Chesapeake Bay and tidal tributaries in Maryland, U.S.A.

DATE INTERVALS:
The Maryland Chesapeake Bay Long-Term Benthic Monitoring and Assessment Program was implemented in July 1984. This web site currently serves data from July 15, 1995 through September 2020 (summer only). Data from previous years and spring sampling (see below) can be obtained by contacting the Program Lead Scientist (see above), or from the Chesapeake Bay Program Data Hub:

http://www.chesapeakebay.net/data

STUDY DESIGN:
The sampling design of this survey changed several times to accommodate changes in the State of Maryland's objectives for this program. See the history of the benthic monitoring program at this web site: http://www.baybenthos.versar.com/history.htm.

With the current design (July 1994 to present), two types of sites are sampled: (1) fixed sites sampled to identify temporal trends and (2) spatially random sites sampled to assess bay-wide benthic community status. Although the site selection criteria for random sites has changed since 1994, sample collection and laboratory methods have not changed significantly. Fixed sites were sampled twice a year through 2008, in May and in late August or September. From 2009 onwards, fixed sites are sampled once a year in late August or September. Random sites are sampled once a year in late August or September at a new set of locations every year. Three replicate sediment samples for benthos are collected at each of 27 fixed sites with gear used since 1984. One sample is collected at each randomly selected site using a Young grab with a surface area of 440 cm^2. Twenty five random samples per sampling stratum are collected every year for a total of 150 samples in six strata. Samples are sieved on a 0.5-mm screen and preserved in the field.

Site selection, strata, and the name, position, and physical characteristics of fixed sites can be found in the QAPP at this Data Dictionary web site location:
http://www.baybenthos.versar.com/data.htm

VARIABLE NAMES AND DESCRIPTIONS FOR DATA FILES:

MARYLAND BENTHIC PROGRAM SAMPLING EVENT RECORD
File: MDBE_EV.TXT

Name	Description
STATION	Sampling Station
SAMPLE_DATE	Sampling Date (YYYY-MM-DD)
SAMPLE_TIME	Time of Station Positioning/initial sampling (HH:MM)
STRATUM	Sampling Stratum or Tributary Designation (see below for strata)
LATITUDE	Latitude (decimal degrees)
LONGITUDE	Longitude (negative decimal degrees)
LL_DATUM	North American Datum Code

SITE_TYPE	Sampling Site Type (Fixed, Random)
TOTAL_DEPTH	Bottom Depth of Station (meters)
SOURCE	Data Collection Institution
YEARCODE	Sampling Year Code (YY/YY, years bracketing the funding period, July 1-June 30)
CRUISENO	Sampling Cruise Number (1 =Summer, 2 =Spring)
STAEQ85	Pre-1989 Station Designation
STAEQ89	Post-1989 Station Designation
SAMP_TYPE	Sample Collection Type (F =Fixed, M =Bay-wide Random)

MARYLAND BENTHIC PROGRAM SAMPLE COLLECTION RECORD
File: MDBE_SMP.TXT

Name	Description
STATION	Sampling Station
SAMPLE_DATE	Sampling Date (YYYY-MM-DD)
SAMPLE_NUMBER	Sample Replicate Number
GMETHOD	Gear Method Code (BC-PH =Post-hole digger; BC-WC =Wildco box corer; PP =Petite Ponar; VV-YM =Van Veen-modified Young grab)
NET_MESH	Screen Mesh Opening (millimeter)
PENETR	Sampling Gear Penetration Depth (centimeters)
SER_NUM	Source Sample Serial Number
SOURCE	Data Collection Institution
YEARCODE	Sampling Year Code (YY/YY, years bracketing the funding period, July 1-June 30)
CRUISENO	Sampling Cruise Number (1 =Summer, 2 =Spring)
STAEQ85	Pre-1989 Station Designation
STAEQ89	Post-1989 Station Designation

MARYLAND BENTHIC PROGRAM WATER QUALITY DATA RECORD
File: MDBE_WQ.TXT

Name	Description
STATION	Sampling Station
SAMPLE_DATE	Sampling Date (YYYY-MM-DD)
SAMPLE_NUMBER	Sample Replicate Number
SAMPLE_DEPTH	Sample Collection Water Depth (meters)
PARAMETER	Sampling Parameter (CONDUCT, DO, DO_PSAT, PH, SALINITY, WTEMP, see below)
VALUE	Sampling Parameter Value
UNITS	Reporting Units of Value
SOURCE	Data Collection Institution

YEARCODE	Sampling Year Code (YY/YY, years bracketing the funding period, July 1-June 30)
CRUISENO	Sampling Cruise Number (1 =Summer, 2 =Spring)
STAEQ85	Pre-1989 Station Designation
STAEQ89	Post-1989 Station Designation
SAMP_TYPE	Sample Collection Type (F =Fixed, M =Bay-wide Random)

MARYLAND BENTHIC PROGRAM SEDIMENT DATA RECORD
File: MDBE_SED.TXT

Name	Description
STATION	Sampling Station
SAMPLE_DATE	Sampling Date (YYYY-MM-DD)
SAMPLE_NUMBER	Sample Replicate Number
PARAMETER	Sampling Parameter (MOIST, SAND, SILTCLAY, TC, TIC, TN, TOC, see below)
VALUE	Sampling Parameter Value
UNITS	Reporting Units of Value
SOURCE	Data Collection Institution
YEARCODE	Sampling Year Code (YY/YY, years bracketing the funding period, July 1-June 30)
CRUISENO	Sampling Cruise Number (1 =Summer, 2 =Spring)
STAEQ85	Pre-1989 Station Designation
STAEQ89	Post-1989 Station Designation
SAMP_TYPE	Sample Collection Type (F =Fixed, M =Bay-wide Random)

MARYLAND BENTHIC TAXONOMIC AND ABUNDANCE DATA RECORD
File: MDBE_TX.TXT

Name	Description
STATION	Sampling Station
SAMPLE_DATE	Sampling Date (YYYY-MM-DD)
SAMPLE_NUMBER	Sample Replicate Number
SPEC_CODE	Agency Taxon Code
LBL	Label or Taxon Name
TSN	ITIS Taxon Serial Number
PARAMETER	Sample Parameter (COUNT, see below)
VALUE	Sample Parameter Value
UNITS	Reporting Units of Value
SOURCE	Data Collection Institution

GMETHOD	Gear Method Code (BC-PH =Post-hole digger; BC-WC =Wildco box corer; PP =Petite Ponar; VV-YM =Van Veen-modified Young grab)
NET_MESH	Screen Mesh Opening (millimeter)
SKIP	Skip Species Count Indicator (see below)
YEARCODE	Sampling Year Code (YY/YY, years bracketing the funding period, July 1-June 30)
CRUISENO	Sampling Cruise Number (1 =Summer, 2 =Spring)
STAEQ85	Pre-1989 Station Designation
STAEQ89	Post-1989 Station Designation
SAMP_TYPE	Sample Collection Type (F =Fixed, M =Bay-wide Random)

MARYLAND BENTHIC BIOMASS DATA RECORD
File: MDBE_BM.TXT

Name	Description
STATION	Sampling Station
SAMPLE_DATE	Sampling Date (YYYY-MM-DD)
SAMPLE_NUMBER	Sample Replicate Number
SPEC_CODE	Agency Taxon Code
LBL	Label or Taxon Name
TSN	ITIS Taxon Serial Number
PARAMETER	Sample Parameter (AFDW, see below)
VALUE	Sample Parameter Value
VALUE_TYPE	Actual or Estimated (W =Actual Ash-Free Dry Weight)
UNITS	Reporting Units of Value
SOURCE	Data Collection Institution
GMETHOD	Gear Method Code (BC-PH =Post-hole digger; BC-WC =Wildco box corer; PP =Petite Ponar; VV-YM =Van Veen-modified Young grab)
NET_MESH	Screen Mesh Opening (millimeter)
YEARCODE	Sampling Year Code (YY/YY, years bracketing the funding period, July 1-June 30)
CRUISENO	Sampling Cruise Number (1 =Summer, 2 =Spring)
STAEQ85	Pre-1989 Station Designation
STAEQ89	Post-1989 Station Designation
SAMP_TYPE	Sample Collection Type (F =Fixed, M =Bay-wide Random)

MARYLAND BENTHIC INDEX OF BIOTIC INTEGRITY RECORD
File: MDBE_IBI.TXT

Name	Description
STATION	Sampling Station
SAMPLE_DATE	Sampling Date (YYYY-MM-DD)
SOURCE	Data Collection Institution
YEARCODE	Sampling Year Code (YY/YY, years bracketing the funding period, July 1-June 30)
SITE_TYPE	Sampling Site Type (Fixed, Random)
IBI_SALZONE	Bottom Salinity Class (TF =Tidal Fresh, O =Oligohaline, LM =Low Mesohaline, HM =High Mesohaline, P =Polyhaline, see below)
IBI_BOTTOM_TYPE	Sediment Type (M =mud, S =sand)
STAEQ85	Pre-1989 Station Designation
STAEQ89	Post-1989 Station Designation
SAMPLE_NUMBER	Sample Replicate Number
IBI_SCORE	Benthic Index of Biotic Integrity Value for Sample
AVE_IBI_SCORE	Fixed Station Replicate Averaged Benthic Index of Biotic Integrity Value
IBI_PARAMETER	Benthic Index of Biotic Integrity Parameter (PCT_CAR_OMN, PCT_DEPO, PCT_PI_ABUND, PCT_PI_BIO, PCT_PI_F_ABUND, PCT_PI_O_ABUND, PCT_PS_ABUND, PCT_PS_BIO, PCT_PS_O_ABUND, PCT_TANYPODINI, SW, TOLERANCE, TOT_ABUND, TOT_BIOMASS, see below)
VALUE	Benthic Index of Biotic Integrity Parameter Value
SCORE	Benthic Restoration Goal Score for Parameter
R_DATE	Benthic Index of Biotic Integrity Run Date

SAMPLING STRATUM OR TRIBUTARY DESIGNATION:
Probability sites are allocated according to a stratified random sampling scheme designed to produce an annual estimate of area meeting the Restoration Goals for the tidal waters (>1 m MLLW) of the Maryland Chesapeake Bay as well as estimates for six subdivisions or strata. Samples are allocated equally among strata. Regions of the Maryland bay mainstem deeper than 12 m are not included in the sampling strata because these areas are subjected to summer anoxia and have been found to be azoic. The following are the sampling strata (see QAPP for a map of strata):

MET = Maryland Eastern Tributaries
MMS = Maryland Mainstem
MWT = Maryland Upper Western Tributaries
PMR = Potomac River

PXR = Patuxent River
UPB = Maryland Upper Bay

Fixed stations, which are not part of these strata, are designated as HIS = Historical

LIST OF PARAMETERS AND METHODS:

Parameter:	**AFDW** (Taxon ash free dry weight in grams)
Collection Method:	Benthic grab (220 cm^2 surface area Wildco box corer, 250 cm^2 Petite Ponar, 440 cm^2 Young grab) or 250 cm^2 surface area post-hole digger. Contents sieved through 0.5-mm screen and preserved in the field.
Sample Preservatives:	10% buffered formalin with Rose Bengal, transferred to 70% ethanol after 5-8 months.
Sample Storage:	Plastic bottles until commencement of processing
Laboratory Technique:	Since 1994, ash-free dry weight biomass is measured directly for each species (with the exceptions listed below) by drying the organisms to a constant weight at 60°C and ashing in a muffle furnace at 500°C for four hours and re-weighing (ash weight). The difference between dry weight and ash weight is the ash-free weight. Because oligochaetes and chironomids require slide mounting for identification, species-specific biomass for Oligochaeta and Chironomidae is not provided except for *Tubificoides* spp., *Branchiura sowerbyi*, and *Coelotanypus* spp., which do not require slide mounting for identification. Bivalves are crushed to open the shells and expose the animal to drying and ashing (shells included).

Parameter:	**COUNT**
Collection Method:	Benthic grab (220 cm^2 surface area Wildco box corer, 250 cm^2 Petite Ponar, 440 cm^2 Young grab) or 250 cm^2 surface area post-hole digger. Contents sieved through 0.5-mm screen and preserved in the field. See QAPP for detail on where the various types of benthic samplers are used.
Sample Preservatives:	10% buffered formalin with Rose Bengal transferred to 70% ethanol after sorting.
Sample Storage:	Plastic bottles until commencement of processing
Laboratory Technique:	Most organisms are separated from the detritus in gridded petri dishes and sorted into major taxa using binocular dissecting microscopes. After sorting, the organisms are stored in 70% ethanol and subsequently identified to the lowest possible taxonomic level (usually species) and counted. Fragments without heads are eliminated from the counts but included in biomass determinations. Oligochaetes and chironomids are mounted on microscope slides, examined under a compound microscope, and identified to genus and species following procedures based upon currently accepted practices in benthic ecology. If the number of oligochaetes or chironomids in a sample is between 20 and 300 individuals, the sample is split and approximately 50% of the

specimens are mounted. The remaining portion is saved and used in biomass determinations. The sample is split by evenly spreading the specimens in a gridded tray and selecting half of the total number of grids at random. If the number of individuals is greater than 300, grids are selected randomly until 150 specimens are mounted. Total taxonomic counts for each oligochaete and chironomid species are adjusted by the proportion of the total number of specimens mounted in the sample.

Parameter: **CONDUCT** (Conductivity in umho/cm, equivalent to uS/cm)
Collection Method: Hydrolab DataSonde 4a four graphite electrode cell (open-cell design), or YSI-6600 and YSI EXO2 four nickel electrode cell with automatic temperature compensation.
Sample Preservatives: N/A
Sample Storage: N/A
Laboratory Technique: N/A

Parameter: **DO** (Dissolved oxygen in ppm., equivalent to mg/l)
DO_PSAT (Dissolved oxygen percent saturation)
Collection Method: Hydrolab DataSonde 4a membrane-design DO sensor, YSI 6600 Rapid Pulse, or YSI EXO2 optical sensor with automatic temperature and salinity compensation.
Sample Preservatives: N/A
Sample Storage: N/A
Laboratory Technique: N/A

Parameter: **MOIST** (Sediment moisture content in percent)
Collection Method: One sediment sub-sample of approximately 120 ml is taken from the surface of a benthic grab for percent silt-clay, sand, and moisture determination.
Sample Preservatives: None
Sample Storage: Frozen until processing
Laboratory Technique: Weight loss on drying for at least 24 hr at 60^{o} C

Parameter: **PH** (pH of sample)
Collection Method: Hydrolab DataSonde 4a, YSI 6600, or YSI EXO2 combined glass pH and reference sensor automatically compensated for temperature.
Sample Preservatives: N/A
Sample Storage: N/A
Laboratory Technique: N/A

Parameter: **SALINITY** (Salinity in practical salinity units, equivalent to ppt)
Collection Method: Hydrolab DataSonde 4a four graphite electrode cell (open-cell design), or YSI 6600 and YSI EXO2 four nickel electrode cell with automatic temperature compensation.
Sample Preservatives: N/A

Sample Storage: N/A
Laboratory Technique: N/A

Parameter: **SAND** (Sand content in percent by weight)
Collection Method: One sediment sub-sample of approximately 120 ml is taken from the surface of a benthic grab for percent silt-clay, sand, and moisture determination.
Sample Preservatives: None
Sample Storage: Frozen until processing
Laboratory Technique: Sand is separated from silt-clay particles (<62.5 um) by wet sieving, and the percent sand fraction is determined by weighing of the dry sand.

Parameter: **SILTCLAY** (Silt-clay content in percent by weight)
Collection Method: One sediment sub-sample of approximately 120 ml is taken from the surface of a benthic grab for percent silt-clay, sand, and moisture determination.
Sample Preservatives: None
Sample Storage: Frozen until processing
Laboratory Technique: Silt-clay is separated from sand by wet sieving through a 62.5 um screen, and the percent silt-clay fraction is determined by pipette and weighing of the dry mud.

Parameter: **TC** (Total carbon content in percent)
Collection Method: One sediment sub-sample (additional to the silt-clay sediment sub-sample) of approximately 120 ml is taken from the surface of a benthic grab for sediment carbon and nitrogen analysis.
Sample Preservatives: None
Sample Storage: Frozen until processing
Laboratory Technique: Combustion at high temperature (975^{o} C) in a carbon analyzer (Exeter Analytical, Inc., CE-440 Elemental Analyzer) and subsequent measurement of the carbon dioxide produced by thermal conductivity detection. Prior to combustion, each sample is homogenized and oven-dried. No acid is applied.

Parameter: **TIC** (Total inorganic carbon content in percent)
Collection Method: One sediment sub-sample (additional to the silt-clay sediment sub-sample) of approximately 120 ml is taken from the surface of a benthic grab for sediment carbon and nitrogen analysis.
Sample Preservatives: None
Sample Storage: Frozen until processing
Laboratory Technique: Ashing in a muffle furnace at a low temperature of 500^{o}C results in the removal of organic carbon. The inorganic carbon remaining in the ash is then injected in a carbon analyzer (Exeter Analytical, Inc., CE-440 Elemental Analyzer) and combusted at high temperature (975^{o} C). The carbon dioxide produced during combustion is measured by thermal

conductivity detection. Prior to ashing, each sample is homogenized and oven-dried. No acid is applied.

Parameter:	**TN** (Total nitrogen content in percent)
Collection Method:	One sediment sub-sample (additional to the silt-clay sediment sub-sample) of approximately 120 ml is taken from the surface of a benthic grab for sediment carbon and nitrogen analysis.
Sample Preservatives:	None
Sample Storage:	Frozen until processing
Laboratory Technique:	Combustion at high temperature in a Exeter Analytical, Inc., CE-440 Elemental Analyzer. The nitrogen concentration is measured by thermal conductivity against a reference cell after all the carbon and hydrogen in the combustion chamber is removed.

Parameter:	**TOC** (Total organic carbon content in percent)
Collection Method:	One sediment sub-sample (additional to the silt-clay sediment sub-sample) of approximately 120 ml is taken from the surface of a benthic grab for sediment carbon and nitrogen analysis.
Sample Preservatives:	None
Sample Storage:	Frozen until processing
Laboratory Technique:	TOC is determined by performing separate TC and TIC analyses and subtracting the results.

Parameter:	**WTEMP** (Water temperature in deg. C)
Collection Method:	Thermistor attached to Hydrolab DataSonde 4a, YSI 6600, or YSI EXO2 sonde.
Sample Preservatives:	N/A
Sample Storage:	N/A
Laboratory Technique:	N/A

IBI_PARAMETER	**DESCRIPTION**
PCT_CAR_OMN	Percent abundance of carnivore and omnivores
PCT_DEPO	Percent abundance of deep-deposit feeders
PCT_PI_ABUND	Percent abundance of pollution-indicative taxa
PCT_PI_BIO	Percent biomass (AFDW) of pollution-indicative taxa
PCT_PI_F_ABUND	Percent abundance of tidal fresh pollution-indicative taxa
PCT_PI_O_ABUND	Percent abundance of oligohaline pollution-indicative taxa
PCT_PS_ABUND	Percent abundance of pollution-sensitive taxa
PCT_PS_BIO	Percent biomass (AFDW) of pollution-sensitive taxa
PCT_PS_O_ABUND	Percent abundance of oligohaline pollution-sensitive taxa
PCT_TANYPODINI	Tanypodinae to Chironomidae percent abundance ratio
SW	Shannon-Wiener species diversity index (log-base =2)
TOLERANCE	Pollution Tolerance Score
TOT_ABUND	Total species abundance (number per meter squared)
TOT_BIOMASS	Total species biomass (grams AFDW per meter squared)

IBI_SALZONE	DESCRIPTION	RANGE (PSU)
TF	Tidal freshwater	0-0.5
O	Oligohaline	≥0.5-5
LM	Low mesohaline	≥5-12
HM	High mesohaline	≥12-18
P	Polyhaline	≥18

IBI_BOTTOM_TYPE	DESCRIPTION	RANGE (% SILT-CLAY)
M	Mud	>40
S	Sand	0-40

THE SKIP VARIABLE OF THE BENTHIC TAXONOMIC AND ABUNDANCE DATA RECORD:

In counting the number of taxa present in a sample, general taxonomic designations at the generic, familial, and higher taxonomic levels are dropped if there is one valid lower-level designation for that group. For example, if both *Leitoscoloplos* sp. and *Leitoscoloplos fragilis* have been identified in one sample, *Leitoscoloplos* sp. is skipped when counting the number of taxa. Skip codes are used to track these general taxonomic designations.

END OF THE DATA DICTIONARY

ATTACHMENT 4
PROCEDURE MODIFICATION TRACKING FORM

CHESAPEAKE BAY MONITORING PROGRAM
PROCEDURE MODIFICATION TRACKING FORM

PMTF # _____ **☐ APPROVED ☐ DENIED**

This form is used to request approval for modifications and to document approved modifications made to Chesapeake Bay Program Office procedures or methods. It is not a substitute for timely contact with the CBPO Quality Assurance Officer or his/her designee, who may be reached at 1-800-968-7229. A detailed method description including the proposed modification must be attached to this form prior to submittal to CBPO.

DATE SUBMITTED **5/24/2011**		DATE APPROVED
REQUESTOR NAME **Roberto J. Llansó**		ORGANIZATION **Versar, Inc**.
NEWLY PROPOSED MODIFICATION [ ]	FIELD-APPROVED MODIFICATION [**x**]	APPROVED BY: DATE:
TYPE OF PROCEDURE / METHOD	SAMPLING [**x**] ANALYTICAL [ ] REPORTING [ ] FIELD MEASUREMENT [ ] OTHER [ ] SPECIFY:	
DURATION	PERMANENT [**x**] EFFECTIVE DATE: **May 2010** TEMPORARY [ ] START DATE: END DATE:	
PROCEDURE/METHOD DESCRIPTION	**Sampling at Fixed Site 022, Baltimore Harbor**	
MODIFICATION DESCRIPTION	**Relocation of Fixed Site 022 across the Patapsco River channel, from 39.254051, -76.587317 to 39.25808167, -7659512 due to construction (filling) of the Masonville Dredged Material Containment Facility (See Figure below). The historical site was buried by cobble during the construction of the Masonville DMCF. The new site meets the same habitat criteria as the old site (similar salinity, depth, and sediment composition)**	
JUSTIFICATION FOR MODIFICATION	**Construction of Masonville DMCF at previous historical Site 022**	
ANALYTICAL PARAMETERS THAT MAY BE AFFECTED BY THIS CHANGE	**Abundance and biomass of organisms, but probably no parameters will be affected given similar habitat criteria and proximity of the new site to the old one**	
AFFECTED QA PLAN(S) (TITLE, REVISION, & DATE)	**Chesapeake Bay Water Quality Monitoring Program Long-Term Benthic Monitoring And Assessment Component Quality Assurance Project Plan 2011-2012, 24 May 2011**	
AFFECTED CRUISE(S)	**Summer cruise, Fixed long-term Site 022**	
PMTF COMPLETED BY	NAME:	DATE:

STATE APPROVAL: NAME _____ TITLE _____
SIGNATURE _____ DATE _____

CBPO APPROVAL: NAME _____ TITLE _____
SIGNATURE _____ DATE _____

CHESAPEAKE BAY MONITORING PROGRAM
PROCEDURE MODIFICATION TRACKING FORM

PMTF # _____ **☐ APPROVED ☐ DENIED**

This form is used to request approval for modifications and to document approved modifications made to Chesapeake Bay Program Office procedures or methods. It is not a substitute for timely contact with the CBPO Quality Assurance Officer or his/her designee, who may be reached at 1-800-968-7229. A detailed method description including the proposed modification must be attached to this form prior to submittal to CBPO.

DATE SUBMITTED **5/10/2021**		DATE APPROVED
REQUESTOR NAME **Roberto J. Llansó**		ORGANIZATION **Versar, Inc**.
NEWLY PROPOSED MODIFICATION [ ]	FIELD-APPROVED MODIFICATION [**x**]	APPROVED BY: DATE:
TYPE OF PROCEDURE / METHOD	SAMPLING [**x**] ANALYTICAL [ ] REPORTING [ ] FIELD MEASUREMENT [ ] OTHER SPECIFY: [ ]	
DURATION	PERMANENT [ ] TEMPORARY [x]	EFFECTIVE DATE: START DATE: **September 2020** END DATE: **Cons. dependent**
PROCEDURE/METHOD DESCRIPTION	**Sampling at Fixed Site 047, Potomac River at Morgantown**	
MODIFICATION DESCRIPTION	**Relocation of Fixed Site 047 from 38.3638, -76.9837 to 38.37654, -76.98519 due to construction associated with the Potomac River Route 301 Bridge. The historical site was occupied by a construction crane in 2020. The original site will be re-evaluated in 2021. The new site meets the same habitat criteria as the original site (similar salinity, depth, and sediment composition)**	
JUSTIFICATION FOR MODIFICATION	**Construction of Route 301 Bridge at previous historical Site 047**	
ANALYTICAL PARAMETERS THAT MAY BE AFFECTED BY THIS CHANGE	**Abundance and biomass of organisms, but probably no parameters will be affected given similar habitat criteria and proximity of the new site to the original site**	
AFFECTED QA PLAN(S) (TITLE, REVISION, & DATE)	**Chesapeake Bay Water Quality Monitoring Program Long-Term Benthic Monitoring And Assessment Component Quality Assurance Project Plan 2021-2022, 10 May 2021**	
AFFECTED CRUISE(S)	**Summer cruise, Fixed Long-Term Site 047**	
PMTF COMPLETED BY	NAME:	DATE:

STATE APPROVAL: NAME _____ TITLE _____
SIGNATURE _____ DATE _____

CBPO APPROVAL: NAME _____ TITLE _____
SIGNATURE _____ DATE _____