CHESAPEAKE BAY WATER QUALITY MONITORING PROGRAM LONG-TERM BENTHIC MONITORING AND ASSESSMENT COMPONENT QUALITY ASSURANCE PROJECT PLAN 2002-2003

Prepared for

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FOREWORD

This document, *Chesapeake Bay Water Quality Monitoring Program: Long-Term Benthic Monitoring and Assessment Component, Quality Assurance Project Plan 2002-2003,* was prepared by Versar, Inc. at the request of Dr. Robert Magnien of the Maryland Department of Natural Resources under Cooperative Agreement CA-02-01/07-4-30722-3734 between Versar, Inc., and the University of Maryland Center for Environmental and Estuarine Studies. 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 quality.

TABLE OF CONTENTS

			Page
FOREWORD			ii
1.0	**INTRODUCTION**		1-1
	1.1	BACKGROUND	1-1
	1.2	OBJECTIVES OF THIS DOCUMENT	1-2
	1.3	ORGANIZATION OF THIS DOCUMENT	1-2
2.0	**PROGRAM ORGANIZATION, MANAGEMENT, AND PERSONNEL**		2-1
	2.1	PROGRAM MANAGER	2-1
	2.2	FIELD OPERATIONS CHIEF	2-1
	2.3	LABORATORY MANAGER	2-1
	2.4	DATA MANAGER	2-2
	2.5	GIS COORDINATOR	2-2
	2.6	DOCUMENT PRODUCTION MANAGER	2-2
	2.7	QUALITY ASSURANCE/QUALITY CONTROL FOR SUBCONTRACTORS	2-2
	2.8	QUALIFICATIONS OF TECHNICAL STAFF	2-3
3.0	**FIELD PROGRAM**		3-1
	3.1	PROGRAM OBJECTIVES	3-1
	3.2	SITE SELECTION	3-2
		3.2.1 Fixed Sites	3-2
		3.2.2 Probability Sites	3-2
		3.2.3 Special Sites	3-12
	3.3	CRUISE PREPARATION	3-12
		3.3.1 Vessel, Crew, and Scientific Party Scheduling	3-12
		3.3.2 Site Identification	3-13
		3.3.3 Label and Field Data Sheet Production	3-13
		3.3.4 Equipment coordination	3-13
	3.4	SAMPLING CRUISE	3-14
		3.4.1 Station Location	3-14
		3.4.2 Sampling Failure	3-14
		3.4.3 Water Column Measurements	3-14
		3.4.4 Benthic Samples	3-15
	3.5	POST-CRUISE	3-16
4.0	**LABORATORY PROCESSING**		4-1
	4.1	BIOLOGICAL SAMPLES	4-1
	4.2	SEDIMENT SAMPLES	4-3
	4.3	QUALITY ASSURANCE/QUALITY CONTROL FOR SUBCONTRACTORS	4-3
5.0	**DATA MANAGEMENT**		5-1

TABLE OF CONTENTS (Continued)

Page

6.0 DATA ANALYSIS 6-1
6.1 THE B-IBI AND THE CHESAPEAKE BAY BENTHIC COMMUNITY RESTORATION GOALS 6-1
6.2 FIXED SITE TREND ANALYSIS 6-1
6.3 PROBABILITY-BASED ESTIMATION 6-2

7.0 LITERATURE CITED 7-1

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 an ecological 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; and
- define linkages between water quality and living resources.

The program includes elements to measure water quality, sediment quality, phytoplankton, zooplankton, 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 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 7 Chapters. Chapter 2.0 describes program management, organization, and the areas of responsibility of program personnel. Chapter 3.0 describes the field program including site selection, field measurements, and instrument calibration. Chapter 4.0 provides an overview of laboratory procedures and data quality objectives; specific steps for each procedure are described in the Versar, Inc. Standard Laboratory Procedures Manual. Chapter 5.0 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 6.0 provides an overview of standard statistical and graphical analysis techniques as well as standard products included in reports. Chapter 7.0 is a list of the literature cited.

2.0 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.

2.1 PROGRAM MANAGER

The Project is organized with a Program Manager responsible for all day-to-day activities. The Program 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 Program 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 Program 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 Program Manager functions through five Activity Managers each responsible for different aspects. These Activity Managers are: (1) Field Operations Chief, (2) Laboratory Manager, (3) Data Manager, (4) GIS Manager, and (5) Document Production Manager.

2.2 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.

2.3 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. The Laboratory Manager oversees day-to-day operation of the Laboratory Quality Assurance and Quality Control Program for Versar and subcontractor laboratories.

2.4 DATA MANAGER

The Data Manager ensures that data meet data quality objectives and coordinates many program activities 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. Finally, the Data Manager adds these data to the MD DNR's long-term benthic data base.

2.5 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.

2.6 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.

2.7 QUALITY ASSURANCE/QUALITY CONTROL FOR SUBCONTRACTORS

Versar closely monitors the QA/QC protocols of its subcontractors and ensures and verifies that Versar QA/QC protocols and standards are applied to all work. 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. In addition, random subsamples of material sorted by subcontractors are submitted to Versar for resorting and verification of identifications and counts, in addition to subcontractor QA/QC protocols. Organism identifications are performed by qualified experts and a taxonomic voucher collection is maintained.

2.8 QUALIFICATIONS OF TECHNICAL STAFF

Dr. Roberto Llansó is Versar's Project Manager specializing in marine science and benthic ecology. Dr. Llansó has extensive experience in conducting benthic ecological studies, sediment quality, and water quality assessments. For five years, Dr. Llansó led the long-term 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, the preparation of reports, and the 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 biocriteria in Puget Sound. He was Project Manager for the Washington State Department of Ecology of a study that provided evaluations and recommendations for benthic indices, interpretative methods, and regulatory thresholds. Dr. Llansó was Invited Speaker at an international seminar in Madrid (Spain) on approaches used to evaluate contaminated sediments, including criteria development and sediment management standards, monitoring, and dredging. Dr. Llansó received his Ph.D. in 1990 from the 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 and western Europe. In recent years, Dr. Llansó has been particularly interested in taxonomic standardization issues for which he has founded and incorporated the Northern Association of Marine Invertebrate Taxonomists (NAMIT).

Ms. Lisa Scott, Laboratory Manager, has over 18 years of experience in benthic ecology and specializes in estuarine and freshwater benthic ecology. She has participated in LTB since its inception in 1984 and is thoroughly familiar with the technical activities of the project. She has also participated in benthic invertebrate monitoring projects in Delaware Bay, the Susquehanna River, and several other freshwater and estuarine ecosystems. She manages the Versar biological and sediment laboratories and her project duties include QA/QC of water quality, sediment, and biological data. She assists Dr. Llansó in data analysis, interpretation, and report preparation.

Mr. Fred Kelley, Field Operations Chief, has coordinated LTB field efforts since 1992, and has participated regularly in sampling efforts since 1990. He has participated in many projects monitoring benthos, sediments, and water quality, including the U.S. EPA Environmental Monitoring and Assessment Program (EMAP). Through these projects, he has developed an intimate knowledge of the Maryland Chesapeake Bay and its tributaries. This knowledge has been critical for successful implementation of the LTB baywide random sampling program.

Ms. Jodi Dew has recently been appointed as data analyst and computer programmer at Versar. She serves as LTB data manager, coordinates sample site selection activities between GIS and field staff, maintains data integrity and streamline operations through automated production of field and laboratory data sheets, and automates integration of QA/QC activities. She maintains the LTB data base, incorporating data from field instruments and several different laboratories, and modifying it as necessary due to sampling design changes. Ms. Dew performs statistical analyses of the data and coordinates with Versar's graphics and GIS departments to display these results. She is also involved in data management and analysis for a variety of projects at Versar, including fisheries research and modeling. Ms. Dew holds a Master of Science degree in Fisheries Science from Virginia Polytechnic Institute and State University, and has over two years experience in data compilation, data analysis, database management, computer programming in SAS and Visual Basic, webpage maintenance and design, population dynamics modeling, and fisheries stock assessment. For her M.S. research, Ms. Dew designed a population model using Visual Basic programming language that predicts the probability that a triploid Suminoe oyster population will become self-sustaining and the probability of localized eastern oyster extirpation in the Chesapeake Bay.

Ms. Nancy Mountford and Mr. Tim Morris of Cove Corporation are recognized authorities on the taxonomy and identification of Chesapeake Bay benthic organisms. They have participated in power plant impact studies on benthic biota, including studies of meiobenthic species. 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. Cove Corporation will process benthic samples, as needed.

3.0 FIELD PROGRAM

The field program is supervised by the Field Operations Chief and consists of four phases of activity: (1) site selection, (2) cruise preparation, (3) sampling cruise, and (4) post-cruise. Samples are collected two times each year, in spring and in summer. Only site selection differs between spring and summer; all other phases are similar both seasons.

Three seasonal definitions are used by the program (Table 3-1). 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 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 3-1. Season definitions. Differences are explained in the text.

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

3.1 PROGRAM OBJECTIVES

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

1) To assess 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 3.2.1 below.

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 3.2.2 below.

From time to time, additional objectives are defined and addressed by special sampling programs at special sites, as described in Section 3.2.3 below.

3.2 SITE SELECTION

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

3.2.1 Fixed Sites

The 27 fixed sites (Figure 3-1) are used to identify temporal trends in benthic condition. Most of the sites have been sampled since 1984 (Figure 3-1). They are all sampled in both spring and summer. Sites are defined by geography (within 1 km from a fixed location) and by specific depth and substrate criteria. Table 3-2 is a list of the 27 fixed sites.

3.2.2 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 area meeting the restoration goals for the Maryland Bay, as well as estimates for six subdivisions. Samples are allocated equally among strata (Figure 3-2, Table 3-3). 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, every point of the Maryland Bay bottom deeper than 1 m mean lower low water (MLLW) has a chance of being sampled.

3.2.2.1 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.

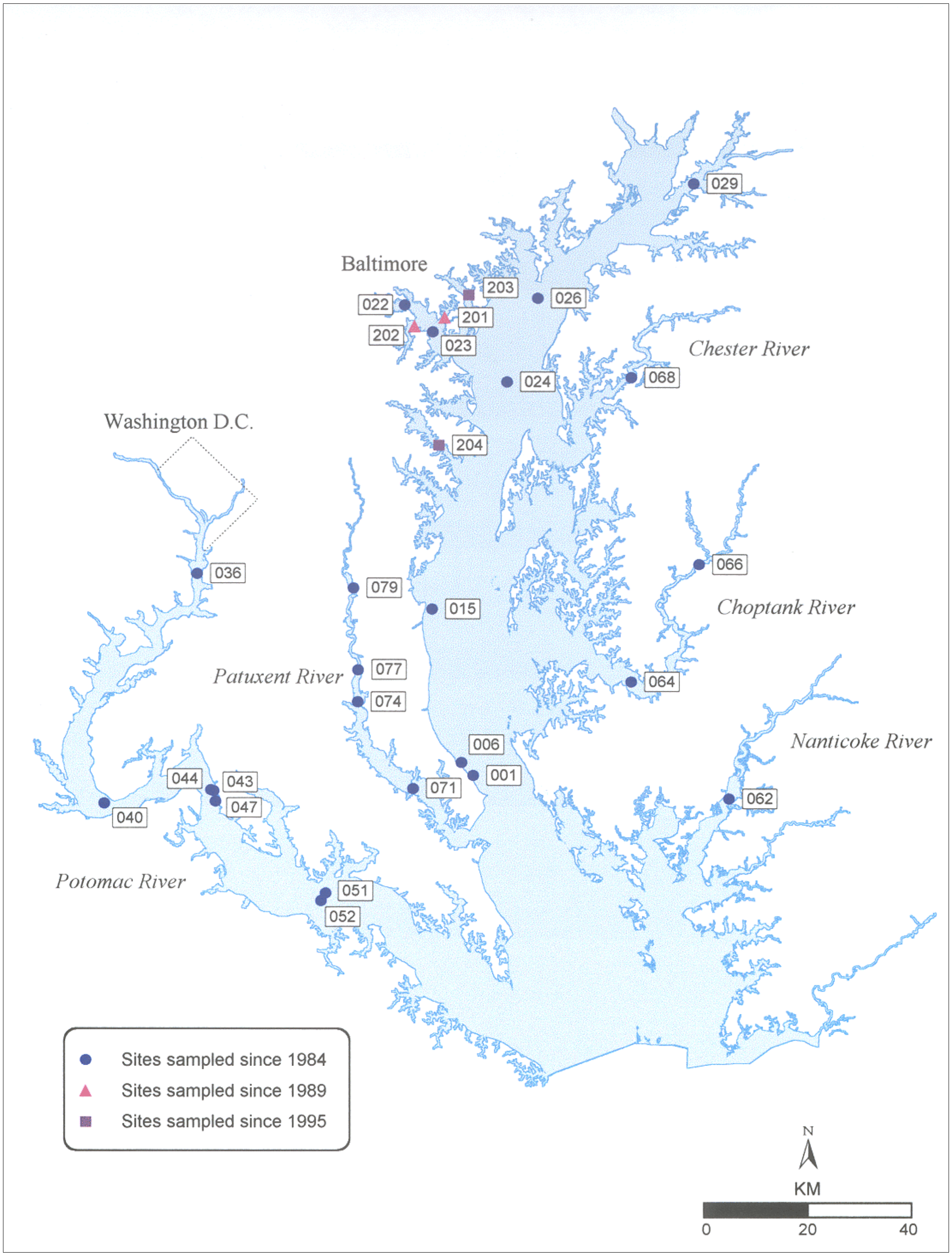

Figure 3-1. Maryland fixed benthic sites.

Table 3-2. Location, habitat (Table 5, Weisberg et al. 1997), sampling gear, and habitat criteria for fixed sites.

Stratum	Sub-Estuary	Habitat	Station	Latitude (NAD 83)	Longitude (NAD 83)	Sampling Gear	Habitat Criteria		
							Depth (m)	Siltclay (%)	Distance (km)
Potomac River	Potomac River	Tidal Freshwater	036	38.769781	77.037531	WildCo Box Corer	< = 5	> = 40	1.0
		Oligohaline	040	38.357458	77.230534	WildCo Box Corer	6.5-10	> = 80	1.0
		Low Mesohaline	043	38.384125	76.989028	Modified Box Corer	< = 5	< = 30	1.0
		Low Mesohaline	047	38.365125	76.984695	Modified Box Corer	< = 5	< = 30	0.5
		Low Mesohaline	044	38.385625	76.995695	WildCo Box Corer	11-17	> = 75	1.0
		High Mesohaline Sand	051	38.205462	76.738020	Modified Box Corer	< = 5	< = 20	1.0
		High Mesohaline Mud	052	38.192297	76.747687	WildCo Box Corer	9-13	> = 60	1.0
Patuxent River	Patuxent River	Tidal Freshwater	079	38.750448	76.689020	WildCo Box Corer	< = 6	> = 50	1.0
		Low Mesohaline	077	38.604452	76.675017	WildCo Box Corer	< = 5	> = 50	1.0
		Low Mesohaline	074	38.547288	76.674851	WildCo Box Corer	< = 5	> = 50	0.5
		High Mesohaline Mud	071	38.395124	76.548844	WildCo Box Corer	12-18	> = 70	1.0

Table 3-2. (Continued)

Stratum	Sub-Estuary	Habitat	Station	Latitude (NAD 83)	Longitude (NAD 83)	Sampling Gear	Habitat Criteria		
							Depth (m)	Siltclay (%)	Distance (km)
Upper Western Tributaries	Patapsco River	Low Mesohaline	023	39.208275	76.523352	WildCo Box Corer	4-7	> = 50	1.0
	Middle Branch	Low Mesohaline	022	39.254940	76.587354	WildCo Box Corer	2-6	> = 40	1.0
	Bear Creek	Low Mesohaline	201	39.234275	76.497184	WildCo Box Corer	2-4.5	> = 70	1.0
	Curtis Bay	Low Mesohaline	202	39.217940	76.563853	WildCo Box Corer	5-8	> = 60	1.0
	Back River	Oligohaline	203	39.275107	76.446015	Young-Grab	1.5-2.5	> = 80	1.0
	Severn River	High Mesohaline Mud	204	39.006778	76.504683	Young-Grab	5.7-7.5	> = 50	1.0
Eastern Tributaries	Chester River	Low Mesohaline	068	39.132941	76.078679	WildCo Box Corer	4-8	> = 70	1.0
	Choptank River	Oligohaline	066	38.801447	75.921825	WildCo Box Corer	< = 5	> = 60	1.0
		High Mesohaline Mud	064	38.590464	76.069340	WildCo Box Corer	7-11	> = 70	1.0
	Nanticoke River	Low Mesohaline	062	38.383952	75.849988	Petite Ponar Grab	5-8	> = 75	1.0

Table 3-2. (Continued)

Stratum	Sub-Estuary	Habitat	Station	Latitude (NAD 83)	Longitude (NAD 83)	Sampling Gear	Habitat Criteria		
							Depth (m)	Siltclay (%)	Distance (km)
Upper Bay	Elk River	Oligohaline	029	39.479615	75.944499	WildCo Box Corer	3-7	> = 40	1.0
	Mainstem	Low Mesohaline	026	39.271441	76.290011	WildCo Box Corer	2-5	> = 70	1.0
		High Mesohaline Mud	024	39.122110	76.355346	WildCo Box Corer	5-8	> = 80	1.0
Mid Bay	Mainstem	High Mesohaline Sand	015	38.715118	76.513677	Modified Box Corer	< = 5	< = 10	1.0
		High Mesohaline Sand	001	38.419956	76.416672	Modified Box Corer	< = 5	< = 20	1.0
		High Mesohaline Sand	006	38.442456	76.443006	Modified Box Corer	< = 5	< = 20	0.5

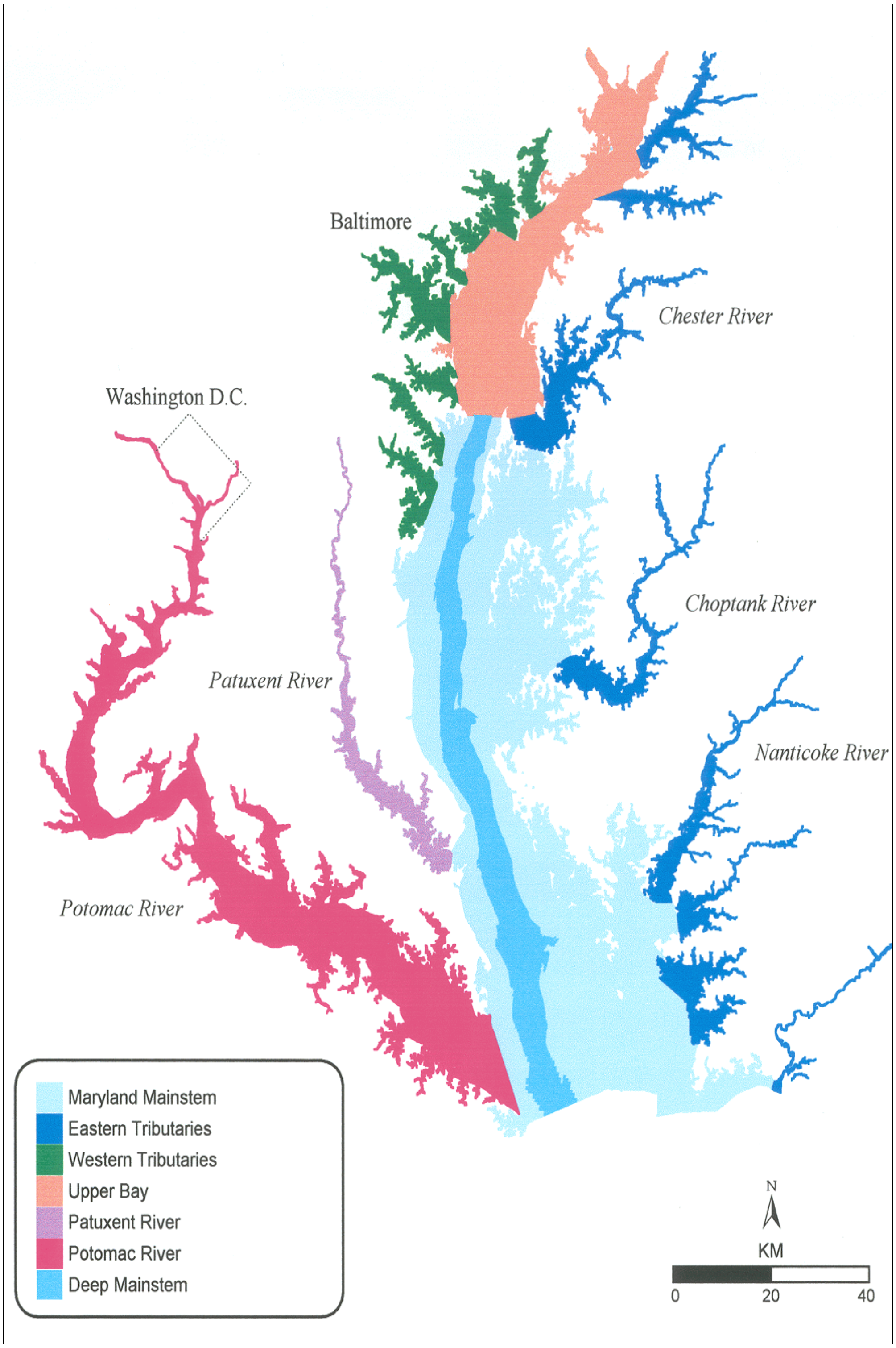

Figure 3-2. Maryland baywide sampling strata.

Table 3-3. 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

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.

3.2.2.2 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 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.

3.2.2.3 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 3-4. 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 Maryland Department of Natural Resource'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 3-4.

3.2.2.4 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.

Table 3-4. 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 3-4. (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

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.

3.2.3 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.

3.3 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.

3.3.1 Vessel, Crew, and Scientific Party Scheduling

Large and small vessels are used by the Maryland Benthic Monitoring and Assessment Component 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.

3.3.1.1 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.

3.3.1.2 Small (Versar) Vessels

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

3.3.2 Site Identification

1) The GIS Coordinator passes a file containing the "top 30" probability site selections for each stratum (Section 3.2.2.4 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; 2002=09). 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.

3.3.3 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.

3.3.4 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.

3.4 SAMPLING CRUISE

3.4.1 Station Location

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

At fixed sites where depth and habitat type have been defined (Table 3-2), 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.

3.4.2 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 at 20 m to 30 m distances 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.

3.4.3 Water Column Measurements

At fixed sites, water column vertical profiles of temperature, conductivity, salinity, dissolved oxygen concentration (DO), and pH are measured using a Hydrolab unit. The profiles consist of water quality measurements at 1 m intervals from surface to bottom at sites 7 m deep or less, and at 3 m intervals, with additional measurements at 1.5 m intervals in the vicinity of the pycnocline, at sites deeper than 7 m. At all other sites, surface and bottom measurements are made. Table 3-5 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 Hydrolab is recalibrated every other day. The instrument is also recalibrated before and after each cruise to determine the amount of drift. The Hydrolab is calibrated according to manufacturer’s specifications. These 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.

Table 3-5. Methods used to measure water quality parameters

Parameter	Method
Temperature	Thermistor attached to Hydrolab H2O or Hydrolab DataSonde 3
Salinity and Conductivity	Hydrolab H2O or Hydrolab DataSonde 3 nickel six-pin electrode-salt water cell block combination with automatic temperature compensation
Dissolved Oxygen	Hydrolab H2O or Hydrolab DataSonde 3 membrane design probe with automatic temperature and salinity compensation
pH	Hydrolab H2O or Hydrolab DataSonde 3 glass pH electrode and standard reference (STDREF) electrode 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.

3.4.4 Benthic Samples

Samples are collected using four kinds of gear depending on the program element and habitat type. At fixed sites (Figure 3-1, Table 3-2), a hand-operated 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 225 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.

At each site, sample volume and penetration depth are measured for all samples; Wildco and hand-operated 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.

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 3-3 provides an overview of QA/QC for biological sample collection.

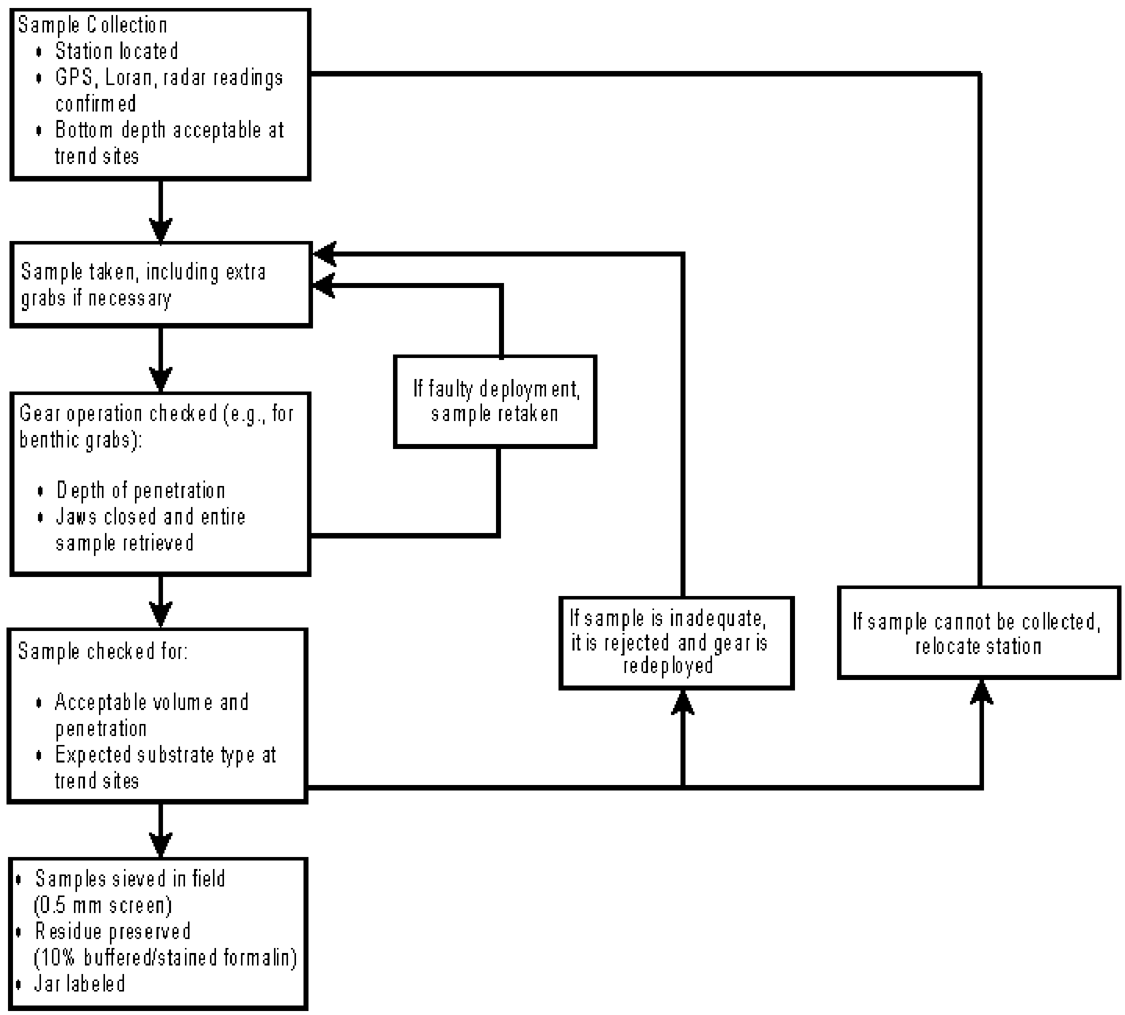

Figure 3-3. QA/QC for biological sample collection

Two surface-sediment sub-samples of approximately 120 ml each are collected for grain-size, carbon, and nitrogen analysis from an additional grab sample at each site. They are frozen until processed in the laboratory.

3.5 POST-CRUISE

All instruments are post-calibrated. Data are downloaded from the 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.

4.0 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 are presented in Figure 4-1.

4.1 BIOLOGICAL SAMPLES

Biological samples are tracked using an automated digital sample tracking system, batch transmittal forms, catalog sheets, and bench sheets. This information is used as applicable to track the location and progress of sample processing in the laboratories.

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.

Samples sorted by each technician are resorted on a regular but arbitrary basis to ensure that all organisms are removed from extraneous material (efficiency of 95% is typically considered acceptable). Approximately 10% of all samples processed are resorted for quality assurance. The level of effort expended on resorts depends on the specific needs of each project but typically ranges from 5% to 20%, depending on whether the intent of the resorts is to confirm efficiency levels and support training efforts, or to guarantee a specified efficiency level within defined statistical confidence limits. 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 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 Institute, 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.

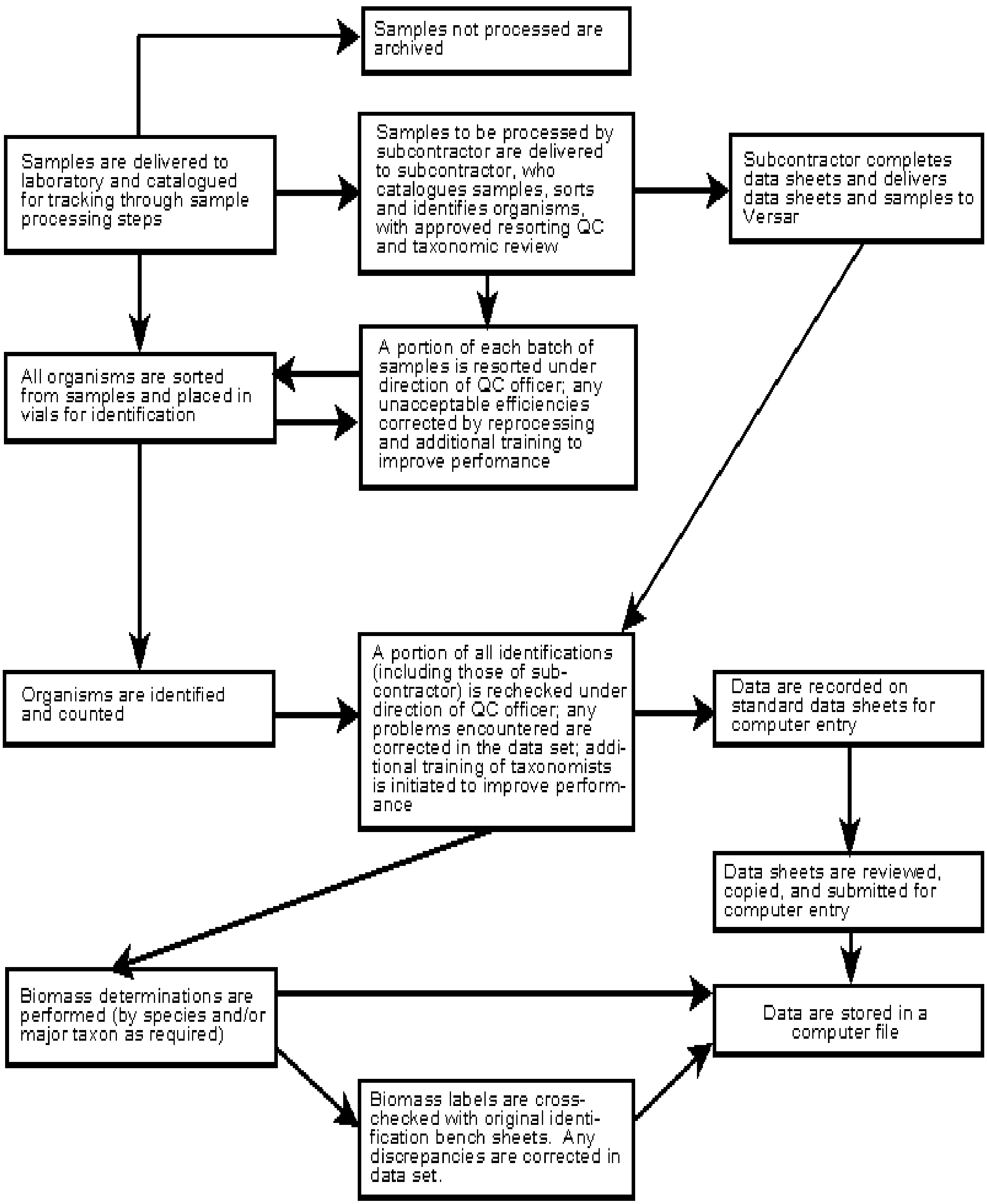

Figure 4-1. QA/QC for biological sample collection and processing

The QA/QC Officer 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.

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.

4.2 SEDIMENT SAMPLES

Silt-clay composition is determined from one of the two sediment sub-samples, and carbon and nitrogen content are determined from the other sediment sub-sample collected at each sampling site.

For silt-clay determination, sand and silt-clay particles are separated by wet-sieving through a 63-μm stainless steel sieve and weighed using the procedures described by the Versar Benthic Laboratory Standard Operating and Quality Control Procedures.

Sediment sub-samples not immediately required for processing are frozen and stored to allow reprocessing for QC or confirmation of questionable results. Ten percent of each sample collection is reprocessed as a QC. Any questionable samples (i.e., values that fall outside of expected ranges) are reprocessed for verification.

Carbon and nitrogen content of dried sediments prepared according to the Versar Benthic Laboratory Standard Operating and Quality Control Procedures 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. The instrument is an Exeter Analytical Inc., Model CE440 analyzer.

4.3 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. In addition, sorted material is retained for resorting and verification of identifications and counts. Organism identifications are performed by qualified experts and a taxonomic voucher collection is maintained. Versar closely monitors the QA/QC protocols of its subcontractors.

5.0 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 with those of the Virginia Chesapeake Bay Benthic Monitoring Program. An overview of the process is provided in Figure 5-1.

All data taken in the field or lab are recorded on standard data forms designed for the project. All data to be entered into electronic files are accompanied by a Data Keypunching Request Form which includes information on the project, data format, and any special instructions. Original data forms are copied before they are sent for keypunching, and the originals are maintained in project files.

Data are entered twice by different data entry operators to provide keypunch verification. When data are entered, 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 5-1 are flagged for special attention. Data output files are then verified against original data sheets to ensure that the computer file is complete and correct. A catalog of data entry formats is maintained, referenced by number, and associated with specific standard data sheets. A 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 Laboratory Manager 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. After a data file is edited, data validation procedures are repeated. All files on the computer system are regularly backed up. 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 Chesapeake Bay Program. These data are stored in accordance with the EPA Benthic Monitoring Data Dictionary.

Table 5-1. 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	$ 1, # 4
Gear Code	In List
Conversion Factor	Match with Gear Code
Serial Number	Cruise Serial Number Range
Depth	> 0, # 35 m
Bottom Depth	$ Depth
Salinity	$ 0, < 25 ppt
Conductivity	$ 0, # 45 mmho
DO	$ 0, # 17 ppm
pH	$ 6.0, # 9.5
Temperature	$ 0, # 29.0 °C

C. SEDIMENT DATA

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

D. TAXONOMIC DATA

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

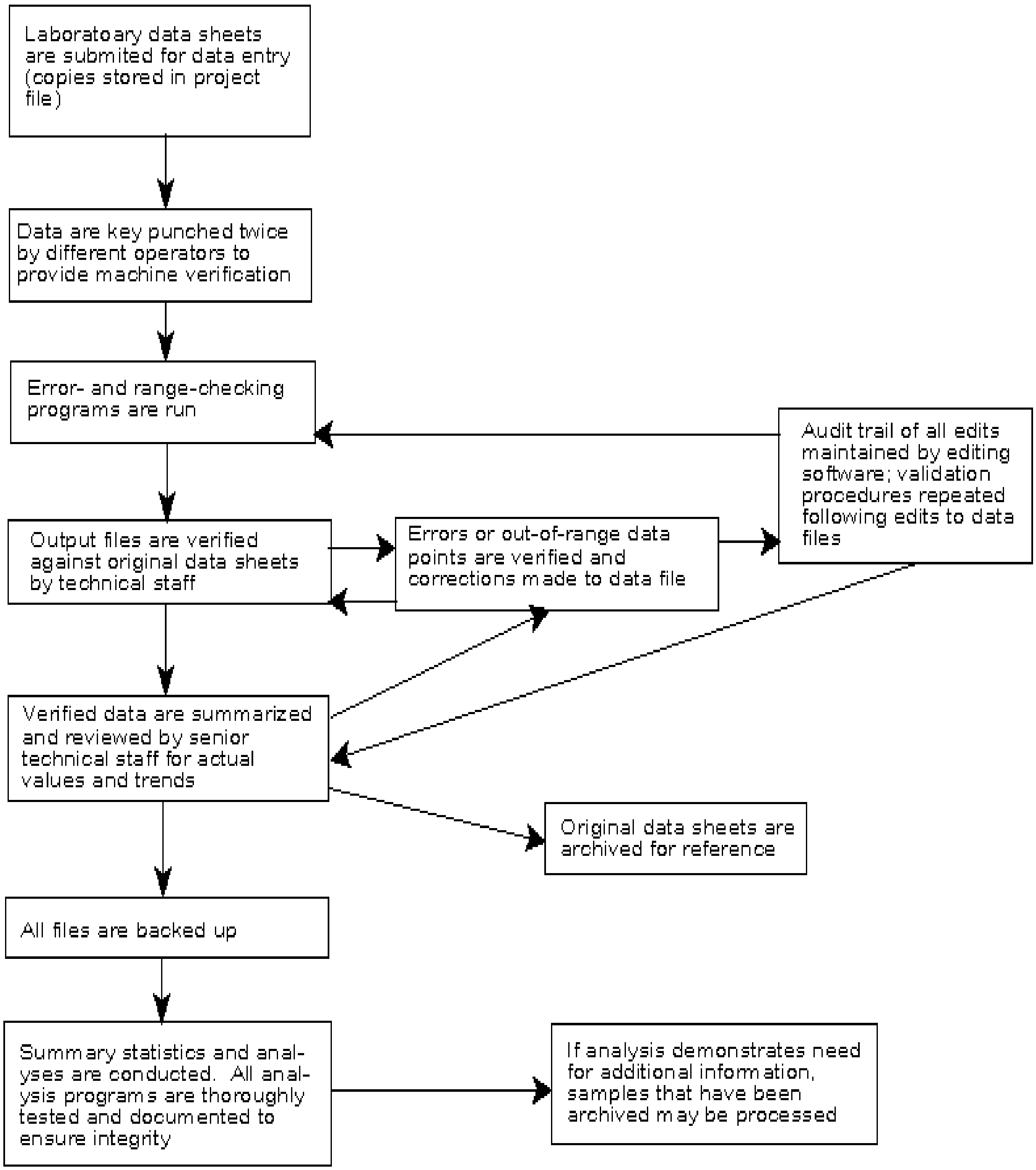

Figure 5-1. QA/QC for data processing

6.0 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.

6.1 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 B-IBI is scaled from 1 to 5, and 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 goal. 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.

6.2 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).

6.3 PROBABILITY-BASED ESTIMATION

The Maryland Bay is divided into six strata (Figure 3-2, Table 3-3). 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:

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

and

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

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

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

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:

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

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.

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