UPPER BAY SURVEY

Final Report to the
Maryland Department of Natural Resources
Annapolis, Maryland 21401

VOLUME II

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PREFACE

Volume II of the Upper Bay Survey final report contains the contributions of the principal investigators in the biological, geological, and physical science fields. Each chapter reports the details and data summations which substantiate the findings. Each chapter is the product of a particular investigator; the words, the thoughts, and the responsibility for the content are his. However, Chapter 1 is an exception.

Chapter 1 was written as an integrating overview. For example, Chapter 6 discusses the biochemistry work, but in Chapter 1, the biochemistry findings and conclusions are integrated as appropriate into the marine biology, microbiology, sedimentology, and hydrology results. Chapter 1 provides the consolidated, multidisciplinary understanding of the entire program, including the objectives of the survey, the rationale in the technical approach, the execution of the project, and a summary of the findings.

In this summary, the pattern of integration is seen clearly to be the pathways of the pollutants through the systems of the upper Chesapeake Bay. The pathways are the key to a clear understanding and ultimate management of the bay. That these pathways are defined more completely and described as never before is the outstanding contribution of this project. It is recommended that this part be read and studied carefully for the best appreciation of the entire major work.

Volume III, Automated Data Processing Operations and Supplementary Data contains the complete set of fundamental data acquired during the survey as well as description and instructions for use of the data base. Volume IV, Numerical Modeling, contains the description and discussion of the upper bay mathematical model developed during the project. As the reader has seen, Volume I, Executive Summary, is an introduction and digest of the entire project including conclusions and recommendations.

At first glance, Chapter 1 of Volume II seems to be largely redundant with Volume I. The reader must recognize that they have distinctly different purposes. Volume I is intended to be the essence of the information contained in the other volumes expressed in uncomplicated language. It is for the busy executive to read; it is for the government administrator and the natural resource manager especially. Chapter 1 of Volume II summarizes the scientific chapters of this volume only, and it is written for the scientist and the project manager.

It is expected that the public official who reads Volume I may make informed judgments based upon its content, but that he will consult with his technical staff for precise definitions. His scientists will have the access to and the ability to digest and interpret Volumes II, III, and IV.

TABLE OF CONTENTS

PREFACE

CHAPTER 1
Multidisciplinary Overview
(T. O. Munson)

CHAPTER 2
Marine Biology
(J. M. Forns)

CHAPTER 3
Microbiology
(R. R. Colwell, G. S. Sayler, and J. D. Nelson, Jr.)

CHAPTER 4
Estuarine Sedimentology
(H. D. Palmer, J. R. Schubel, and W. B. Cronin)

CHAPTER 5
Hydrology and Meteorology
(K. T. S. Tzou)

CHAPTER 6
Biochemistry
(T. O. Munson)

CHAPTER 1

MULTIDISCIPLINARY OVERVIEW

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TABLE OF CONTENTS

Section		Page
1.0	INTRODUCTION	1-1
1.1	Concept	1-1
1.2	Objectives	1-1
2.	OVERVIEW	1-3
2.1	Integration	1-3
2.2	Pathways	1-3
3.	THE SURVEY	1-7
3.1	The Setting	1-7
3.2	The Approach	1-9
3.3	Participants	1-12
4.	RESULTS	1-15
4.1	Hydrology and Meteorology	1-15
4.2	Sedimentology	1-16
4.2.1	Bottom Sediments	1-16
4.2.2	Suspended Sediments	1-16
4.3	Marine Biology	1-17
4.4	Microbiology	1-18
4.5	Numerical Modeling	1-19
4.6	Data Base	1-20

LIST OF FIGURES

Figure		Page
1-1	The Principal Sampling Stations of the Upper Bay Survey	1-10
1-2	Work Organization of the Disciplinary Tasks	1-11

1. INTRODUCTION

1.1 Concept

The concept of the Upper Bay Survey grew directly from the Chester River Study, a program of more limited scope which was completed in the fall of 1972. An objective of that program was to provide the Maryland Department of Natural Resources with environmental and resource management information essential to the local shellfish industry. These studies were directed toward determining the source and fate of chlorinated hydrocarbons and selected trace metals (i.e., Zn, Pb, Cd, Cu, Cr, and Fe) in estuarine waters and sediments. The approach was multidisciplinary. The project as a whole was considered experimental, since it was to encompass many aspects of an estuarine ecosystem with the declared purpose of supporting the practical needs of natural resource managers. Thus, the Chester River Study was a program of focused, applied research rather than a more basic program to extend knowledge in estuarine sciences.

As a result of this program, the probability became clear that the upper Chesapeake Bay was the source of sediment-borne materials considered potentially harmful to the living resources of the Chester River. An extension of the Chester River project encompassing the bay between the Severn and Susquehanna Rivers, became the next logical step in assessing the impacts of man-made substances in the bay's ecosystem. The Upper Bay Survey was begun in December 1973, fortified by incorporating bacteriological studies.

1.2 Objectives

On the basis of knowledge gained during the more limited Chester River Study, a 12-month field program was implemented to assess the nature of the sources, routes, and sinks of chlorinated hydrocarbons and bacteria damaging to aquatic species. Five definitive objectives for the Upper Bay Survey were to:

(1) Determine concentrations, distributions, and sources for chlorinated hydrocarbons (CHC), including pesticides and polychlorinated biphenyls (PCB), and to determine the nature of transport paths, mechanisms, and rates in the Chesapeake Bay's waters, sediments, and organisms.

(2) Determine immediate and longer-term biological consequences of chlorinated hydrocarbons and PCB's on commercially important species.

(3) Determine the distribution of bacteria on sediments and suspended particles of the upper bay, and to perform toxicological studies of the combined effects of pesticides and bacteria on oysters.

(4) Institute numerical models for projecting contaminant distribution relative to the sources and to develop interrelations to biological impacts resulting from changes in the upper Chesapeake Bay's input stresses.

(5) Report results in a format convenient for resource management and in a format for computer storage, retrieval, and augmentation.

2. OVERVIEW

2.1 Integration

Each contributor to the Upper Bay Survey has presented his or her findings in the separate chapters which follow in Volumes II, III, and IV. These are summarized in this chapter under Section 4, Results. The purpose of this section, Overview, is to draw upon these findings for an overall discussion of the rates, routes, sources, sinks, and reservoirs of the chlorinated hydrocarbons and bacteria in the upper Chesapeake Bay. The emphasis in this chapter is to be on a multidisciplinary approach as opposed to the specific science approaches of each of the following chapters. The thread of the approach is the pathways through the upper bay, including:

- Suspended sediment deposition
- Suspended sediments and zooplankton
- Suspended sediments and bacteria
- Suspended sediments and shellfish
- Bottom sediments and infaunal processes
- Baltimore harbor circulation with the upper Chesapeake Bay.
- Predictive model of the process

2.2 Pathways

The Upper Bay Survey, the Chester River Study, and several similar estuarine or marine investigations have shown that the finer grained sediments are enriched in cations, synthetic organic molecules, and bacteria which are adsorbed to the relatively large surface area of the finer particles generally carried in suspension in bay waters. It is, therefore, important to evaluate the transport of fine sediment through the upper bay.

- The major path for the movement of CHC's from the suspended sediment reservoir involves at least one phase of sediment deposition. Total Viable Count (TVC) of bacteria also is higher in the bottom sediments than in the water. The suspended sediment load appears to be generally constant, a factor which on a budget basis requires that an amount equivalent to the annual fresh load from the Susquehanna be deposited. The areas of deposition of fine-grain sediments can be visualized as sinks where the CHC's and bacteria attached to suspended sediments are deposited.
- Over most of the upper bay, the principal route for movement of these materials from the sinks would be through resuspension of the fine-grains by tidal scour. Resuspension may return large numbers of coliforms and fecal streptococci from the sink to the suspended sediment reservoir. In some areas such as Baltimore harbor, the movement out of the sediment sink is slow and there is a resulting accumulation of fine-grain sediments and their associated contaminants. Where these areas of accumulation coincide with navigational channels, maintenance dredging represents a major mechanism for removal of sediments bearing relatively high concentrations of chlorinated hydrocarbons. Both overboard disposal and hopper barge dredging techniques involve resuspension of large quantities of sediments enriched in CHC's, trace metals and bacteria. The impact of such an influx is currently under study by several groups active in Chesapeake Bay studies.
- A pathway for movement of CHC's out of the seston reservoir is via the zooplankton. At any given time, only a small part (less than one percent) of the total CHC's present in the water-column are detectable in the zooplankton standing stock. The movement, therefore, of CHC's into the biological system from the non-biological system is influenced by changes in the concentration of CHC's adsorbed to suspended sediment. The suspended sediment could be considered a reservoir of CHC's with movement into the biological system from this reservoir when the zooplankton blooms occur. The zooplankton concentrated the CHC's five to eight times over the levels found in the suspended sediment fraction. Whatever the details of this pathway, in order to quantify the rate of movement of CHC's via this route, one would need information regarding turnover rates in the plankton community. (Most likely, phytoplankton also would be involved.) A change in Chesapeake Bay conditions increasing the zooplankton population would probably have the effect of increasing the exchange of CHC's from the suspended sediment reservoir into the biological system with potential adverse consequences.
- Higher counts of bacteria associated with suspended sediments were observed at the stations near the Susquehanna River, but the counts decreased southward to Kent Island. Transport gradients are indicated although a sharp correlation between concentrations of bacteria and suspended sediment was not observed.
- Filtering of bay water by shellfish represents another direct route for the passage of CHC's from the suspended sediment reservoir into the biological system. The shellfish concentrated the CHC's many thousands of times higher than the levels in the water column (e.g., PCB, 4,000; chlordane, 30,000; and DDTR, 45,000). Although shellfish clearly are accumulating CHC's, efforts to quantify this route would require more information on the standing crop of shellfish and the population cycles in the shellfish community of the upper bay. Also, an important consideration is the potential transmission of pathogenic microorganisms to human beings via shellfish, as shellfish are known to concentrate bacteria.
- Another route for CHC movement from the sediment sinks into the biological system is through the deposit-feeding infaunal organisms which inhabit the fine-grain sediments. In particular, certain polychaetes are the most abundant macrofauna in silt-clay habitats, including grossly polluted areas in Baltimore harbor. These organisms form a major part of the diet of crabs and certain species of fish in some areas. This pathway is of particular interest because of the direct route to human beings via crabs and fish in commercial and recreational catches.

- Chemical breakdown of CHC's by non-biological processes and by microbial metabolism constitutes a pathway for the movement of CHC's from the bay's system. However, bacterial metabolism studies show that the microbial degradation of the PCB, Aroclor®* 1254, is extremely slow.
- Baltimore harbor appears to provide local sources of PCB and chlordane which cause some of the very high values found in the sediments. The entry of CHC's from aerial fallout and runoff also cause localized high concentrations in the harbor itself. The major route for CHC's out of the harbor would be removal via dredging and via the stratified flow of water containing suspended sediments. But, the levels in the harbor are the same as those in the reservoir of suspended sediments in the bay, except for some elevation in the chlordane.
- A numerical model has been developed which provides a significant forward stride in the capability to examine the source-sink process. The model describes contaminant sources if they also are sediment sources, which is the dominant case in the upper bay. Although tentative at this stage, the model correctly shows the Susquehanna to be a source of sediments, given sediment concentration data over the upper Chesapeake Bay. It further shows areas of deposition and resuspension and a net southward flow. These positive results are based on limited data and interpolative procedures which will require much more ground truth information to refine them. Nonetheless, the difficult problem of modeling vertical transport has been partially solved.

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* A registered trade name of Monsanto Company, St. Louis, Mo.

3. THE SURVEY

3.1 The Setting

The Chesapeake Bay, and particularly that part within Maryland's borders, is a familiar region adjacent to the southern end of the Washington - Boston metropolitan corridor. It consists of a great many areas that are extremely valuable in terms of industrial, recreational, and agricultural land use. Extensive discussion of the related details is beyond the scope of this volume, so the interested reader is referred to two excellent documents: *The Integrity of the Chesapeake Bay,* Publication Number 184 of the Maryland Department of State Planning and the U. S. Army Corps of Engineers publication, *Chesapeake Bay: Existing Conditions Report* of December 1973 (7 Volumes).

The latter report discussed a series of significant aspects of which some of the most pertinent are extracted and presented below as a cameo of the Chesapeake Bay region:

- "Chesapeake Bay is one of the largest estuaries in the world, having a surface area of about 4,400 square miles and a length of nearly 200 miles. Like many coastal plain estuaries, the Bay is a broad, shallow expanse of water varying from 4 to 30 miles in width, but having an average depth of less than 28 feet.
- "Approximately 7.9 million people inhabited the Bay Area in 1970. This total is more than double the 1940 figure and is expected to double again by the year 2020, reaching approximately 16.3 million people.
- "About 80 percent of the people in the Bay Area live in urban areas. In the last several decades, people have tended to move out of the inner cities and rural counties and into the suburban counties.
- "Over 50 percent of the total growth in the Bay Area between 1970 and 2020 is expected to take place in the Washington, D. C., subregion.
- "Approximately 3.3 million people were employed in the Bay Area in 1970, and the unemployment rate was significantly lower than the national average. The per capita income in 1970 for residents of the Bay Area was $3,690 as compared to $3,390 for the United States as a whole.

- "The four major categories of employers in the Bay Area are the Service Sector (26 percent of total employment), the Wholesale and Retail Trade Sector (17 percent), the Manufacturing Sector (16 percent), and the Public Administration Sector (14 percent). The Bay Area percentage for manufacturing is low, though, when compared to a national average of 25 percent. The public administration sector is almost three times the national percentage, primarily due to large numbers of Federal employees in the Washington, D. C. area.
- "Only a little over one-third of the land around the Bay is considered developed and most, or about 87 percent, of this developed land is in agricultural use. The concentration of people and economic activity is further illustrated by the fact that all land used for residential, commercial, and industrial purposes is less than 5 percent of the total land around the Bay.
- "About 5 percent of the land around the Bay is classified as wetlands. Wetlands which are very important to the productivity of the Bay are being lost or threatened by development at an alarming rate.
- "The Bay receives fresh water from a drainage area of 64,160 square miles. The five major rivers, the Susquehanna, Potomac, Rappahannock, York, and James, provide almost 90 percent of the total fresh water flow into the Bay with the Susquehanna alone providing about 50 percent.
- "The physical composition and structure of the earth in the Bay Area varies from the basically flat, sedimentary Atlantic Coastal Plain Province to the more rugged topography of the Piedmont Plateau Province. The Coastal Plain, includes the Eastern Shore of Maryland and Virginia, most of Delaware, and a portion of the Western Shore to the Fall Line where it adjoins the Piedmont Plateau. The Piedmont Plateau then extends westward past the limits of the study area.
- "The Bay Area is underlain by a wedge of sedimentary deposits which contain several excellent water-bearing sands and gravels that serve as a major source of both public and private fresh water throughout much of the Coastal Plain.
- "Industry is by far the largest water user in the Bay Area with a daily use of approximately 1,600 million gallons as compared to 860 and 100 million gallons per day for public and agricultural use, respectively. The major sources of water are the fresh water tributaries of the Bay, ground water and the brackish water of the estuary itself which is used for cooling.
- "The Chesapeake Bay Region offers a wide variety of water-oriented recreational opportunities. In some parts of the region, though, the supply of facilities is not adequate to meet the increasing public demand. The Washington-Baltimore area especially has a shortage of picnicking, camping, and swimming facilities.
- "The Bay Area is served by five major electric utilities plus a number of small companies which together provide about two-thirds of the power consumed in the Region. The generating facilities within the Bay Area include 21 fossil-fueled, three nuclear, and two hydroelectric plants.
- "Water quality conditions in the Bay vary widely due to a variety of factors; proximity to urban areas, type and extent of industrial and agricultural activity, stream-flow characteristics, and the amount and type of upstream land and water usage. Most of the water quality problems occur in the estauries of the Bay's tributaries and not in the Bay proper.
- "Erosion (caused mainly by the daily tidal process, storm-induced waves, and the wakes of passing ships) and the rising of the sea level cause a loss of approximately 450 acres of shoreline land each year.
- "The economic development of the Bay Area has been largely based on the natural transportation network provided by the Bay and its tributaries. A total of about 150 million tons of cargo was shipped on the Bay in 1970 with the majority passing through the major deep water ports of Baltimore and Hampton Roads. Baltimore is basically an importing port with iron ore, automobiles, and heavy metals the primary commodities. Hampton Roads, on the other hand, is an exporting port with coal being the prime export.

- "Certain aquatic plants have become known as "noxious weeds" because they often interfere with man's use and enjoyment of the Bay. The three most common of these are watermilfoil, sea lettuce, and the water chestnut. The extent of these weeds in the Bay has been reduced in recent years by disease and various controls.
- "Because of the variations in salinity levels, the Chesapeake Estuary supports a wide variety of fish life. Generally, finfish reproduce in the low saline waters of the Upper Bay and the upstream portions of the tributaries. On the other hand, the famous blue crab reproduces in the saltier waters at the mouth of the Bay. In addition, some species use the Bay as a spawning area and nursery, then migrate to the ocean for their adult life.
- "In 1970, Chesapeake Bay landings of a wide variety of shellfish and finfish totaled 630 million pounds valued at $41 million. The most important species from a value standpoint are oysters, crabs, clams, menhaden, and striped bass.
- "The marshes, woodlands, and the Bay itself, provide an extremely productive natural habitat for over 2,700 different species. The sheer number of species alone forecasts the complexity of Bay biota in terms of partitioning species to communities and determining functional relationships that will aid in understanding the Bay as an ecosystem."

3.2 The Approach

The Survey objectives have been noted, and the approaches which address these objectives are listed below. It was essential to perform:

- Multidisciplinary work because of the complex scientific interrelationships involved;
- Near-synoptic observations in order to acquire a picture dominated by longer transients such as high and low flows and seasonal effects;
- Bimonthly sampling and measurements of most parameters to achieve the highest data frequency commensurate with cost;
- Sampling at stations where spacing and positions were suitable to the modeling effort (See Figure 1-1);
- Supporting efforts in laboratory analysis to support the synoptic and seasonal sample regime, and;
- Modeling development to support predictive capabilities in resource management.

The above elements are sufficiently self-explanatory to define the scope of the program, but they do not include other aspects which might be considered for a fully comprehensive effort. For example, there are many boundary conditions for which only spot checks could be made. Examples include airborne particulate fallout sources, runoff analysis, and shoreline erosion. Investigation of pathways through higher trophic levels is needed. Although these are of interest, the clear domination of the Susquehanna led to practical limitations in setting program scope. Another boundary is the number of data points; it was obvious that conclusions would have to be drawn from statistics with large standard deviations.

Because of the dynamic nature of the estuarine system, especially in the case of the shallow upper Chesapeake Bay, it is imprudent to employ merely average values of given parameters in describing background conditions. Broad excursions in temperature, salinity, dissolved oxygen, and other water-mass properties occur in very short periods of time, and they may vary dramatically on a seasonal or an annual basis. Similarly, the biomass is characterized by patchiness and instabilities. Thus, investigators should examine the component data points as well as the statistical summaries which generally appear in graphs and tables. These data are provided in Volume III.

The task breakdown shown in Figure 1-2 illustrates the division of effort in executing the above approach. More detailed task descriptions are evident in each of the principal investigator's reports.

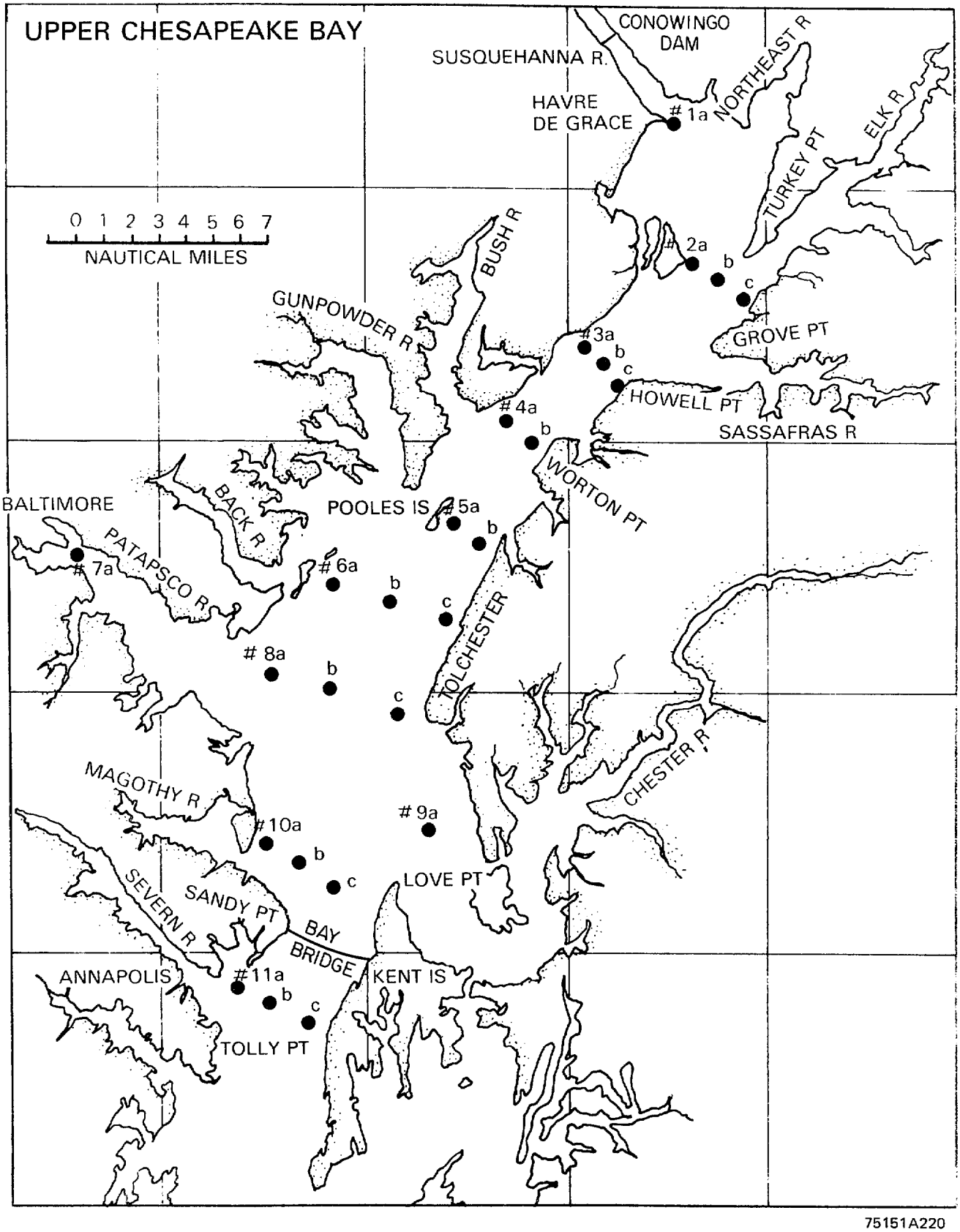

Figure 1-1. The Principal Sampling Stations of the Upper Bay Survey

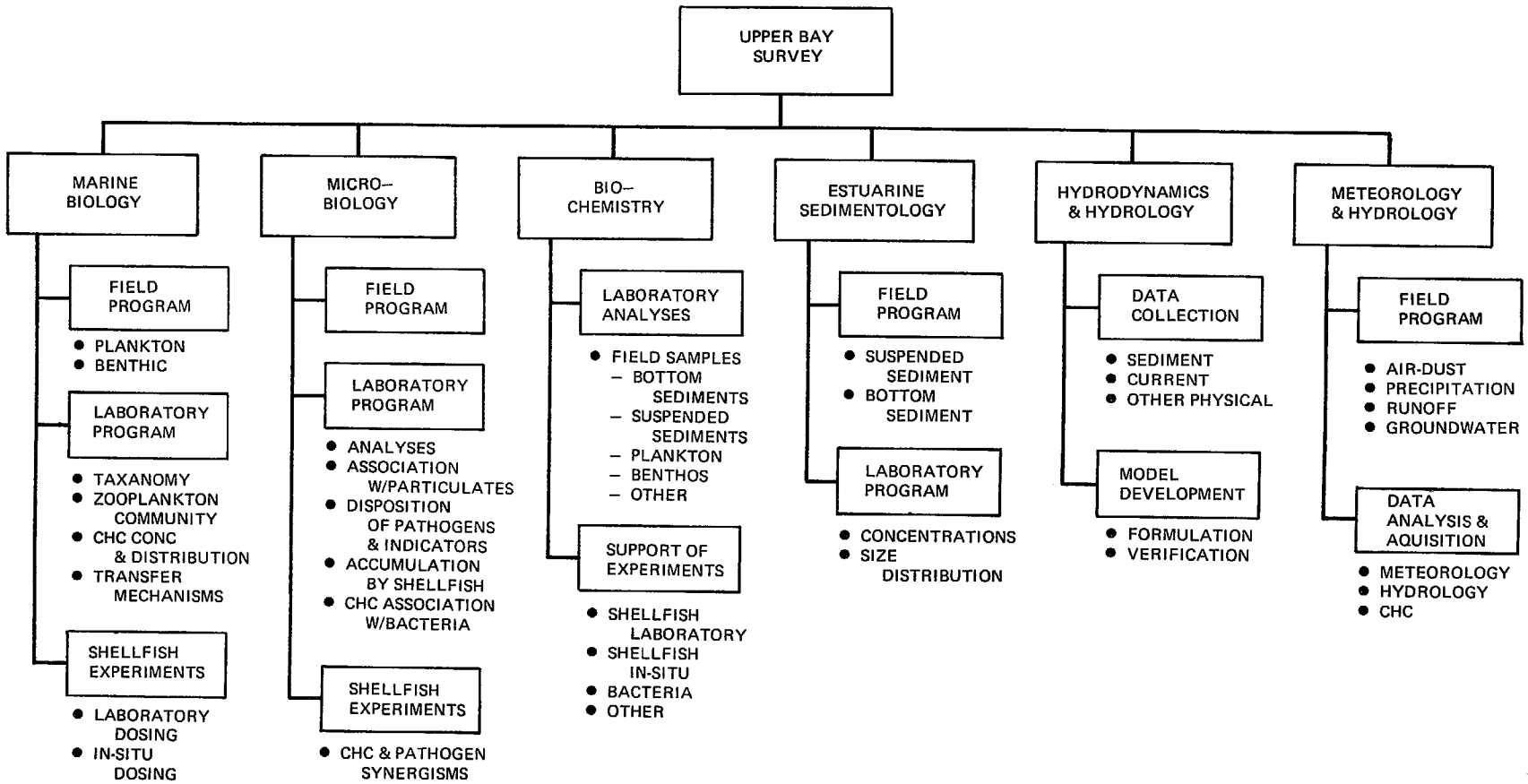

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Figure 1-2. Work Organization of the Disciplinary Tasks

3.3 Participants

The principal investigators were drawn from the Chesapeake Bay Institute (CBI) of The Johns Hopkins University, the Department of Microbiology of the University of Maryland, and the staff of Westinghouse Ocean Research Laboratory. Program and data management were provided by Westinghouse's Oceanic Division, into which Ocean Research Laboratory subsequently merged.

Westinghouse:

Dr. T. O. Munson Project Chief Scientist	Biochemistry
Dr. H. D. Palmer	Estuarine Sedimentology (Bottom)
Dr. K. T. S. Tzou	Hydrology and Meteorology
Mr. J. M. Forns	Marine Biology
Mr. D. K. Ela	Program Manager
Mr. Carleton Rutledge Jr.	Program Engineer
Mr. A. R. Barskis	Data Operations

Chesapeake Bay Institute:

Dr. D. W. Pritchard	Circulation and Modeling
Dr. J. R. Schubel *	Estuarine Sedimentology (Suspended)
Dr. J. R. Hunter	Numerical Modeling

University of Maryland:

Dr. R. R. Colwell	Microbiology
Dr. J. D. Nelson	Microbiology
Dr. G. S. Saylor	Microbiology

The project benefited by the continuing guidance and advice of the Board of Scientific Direction, a peer group formed by the Maryland Department of Natural Resources. Dr. T. Chamberlain, Director of the Chesapeake Research Consortium, was elected chairman of this board to supervise the continuous review of the project on an interactive basis. Thus, the technical progress of the project benefited from the assembled expertise of the board's members, all of whom have extensive experience in Chesapeake Bay research. Members of the Board included:

Chairman:
Dr. Theodore Chamberlain, Director
Chesapeake Research Consortium
The Johns Hopkins University

*Presently at the State University of New York, Stoney Brook, N.Y.

Dr. Rita R. Colwell
Department of Microbiology
University of Maryland

Dr. Eugene Cronin, Director
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Mr. D. K. Ela, Project Manager
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Dr. Peter E. Wagner, Director
Center for Environmental & Estuarine Studies
University of Maryland

Dr. F. Prescott Ward, Director
Ecological Studies
Edgewood Arsenal

4. RESULTS

4.1 Hydrology and Meteorology

Climatologically, during the study-period December 1973 to December 1974, the upper Chesapeake Bay area experienced a mild wet winter, a cool dry summer, and winds less than normal. The Susquehanna River, which contributed 97 percent of the fresh water and suspended sediments to the upper bay, delivered an annual mean flow of 39,911 cfs in 1974. The average yearly flow pattern is typified by a period of overwhelmingly high flow, the spring freshet, usually occuring in March or April. However, in 1974, winter flows were relatively high, and the spring freshet was not the dominant high-flow period because much of the precipitation fell as rain rather than snow.

Air samples collected at a station in the Baltimore harbor area were found to contain chlorinated hydrocarbons—apparently primarily in the vapor phase. In the six air samples analyzed for chlorinated hydrocarbons, including Aroclor® 1254, chlordane (sum of the alpha and gamma isomers), dieldrin, and DDT were found to occur in the ratios (average of all samples) of 4.0 : 1.0 : 0.08 : 0.03. The average PCB (Aroclor® 1254) concentration was five nanograms (10^{-9} grams) per cubic meter of air.

Rainwater samples were collected at two stations in the Baltimore harbor area: (1) Fort Smallwood, a public recreational area away from the heavily industrialized areas, and (2) Sollers Point, a residential area close to a heavily industrialized zone of Baltimore. PCB and the chlorinated pesticides chlordane, toxaphene, DDT, and benzene hexachloride (BHC) were found in the rainwater at both stations. The average PCB for five samples at Fort Smallwood was 0.4 ppt (parts per trillion; i.e., nanograms per liter) and for three samples at Sollers Point was 125 ppt. The average ratio of PCB to chlordane to toxaphene to DDT to BHC (sum of the alpha and gamma isomers) at Fort Smallwood was 0.4 : 0.5 : 30 : 0.3 : 5. The ratio at Sollers Point was 1 : 1 : 0.9 : 0.1 : 0.07.

Six samples of storm runoff were collected from a storm sewer in Morrell Park. Traces of toxaphene were found in one sample, and traces of BHC were found in two samples.

K. T. S. Tzou

4.2 Sedimentology

4.2.1 Bottom Sediments

Much of the bottom sediment of the upper bay consists of fine grained materials of the size of silt and clay particles (less than 62 microns in their longest dimension). Previous studies have shown an inverse relationship between grain size and concentrations of chlorinated hydrocarbons and trace metals; a suite of bottom samples from various sites within the upper Chesapeake Bay have confirmed this relationship. Experiments with bulk samples of bottom sediments in which various size fractions were removed for analyses have proved that: (1) the finer fractions are enriched in PCB's, DDT residues (DDTR), and chlordane and (2) that the Baltimore harbor mud has a significantly higher concentration of chlorinated hydrocarbons than that from other locations sampled during this study.

Deposits of finer-grained materials are concentrated: (1) in areas where currents and turbulence are minimal and (2) in the turbidity maximum where finer materials are re-cycled and deposited through the action of currents associated with tidal scour and net nontidal estuarine flow in a stratified water column. In view of (1) the sustained high levels of chlorinated hydrocarbons on suspended sediments from Station 1A at the mouth of the Susquehanna River and (2) from estimates that deposition is occurring at a rate of at least five to eight millimeters per year, it is concluded that chlorinated hydrocarbons are accumulating in the fine-grained bottom sediments of the upper bay. The chlorinated hydrocarbon levels found in the upper bay sediments were two to three times higher than those observed in the sediments of the Chester River. The sediments from Baltimore harbor were found to contain the greatest amounts of the CHC contaminants with values as high as 3.7 ppm (parts per million) for PCB, 0.082 ppm for chlordane, and 0.19 ppm for DDTR, but it appears that these accumulations are not carried to the Bay through the weak flushing which occurs in the Patapsco River mouth and the harbor.

4.2.2 Suspended Sediments

According to measurements taken at Conowingo Dam during the course of this study, 0.8 million metric tons of suspended sediment passed this point on the lower Susquehanna River. This amount was approximately two-thirds that of the previous year (1973), and an average of about one million metric tons per year may be assumed to enter the upper bay from this source.

The movement of suspended sediments is controlled by two processes. The normal net non-tidal flow regime in effect during most of the year coupled with tidal scour establishes a *turbidity maximum* near the head of the bay, and suspended sediments are trapped by a re-cycling of bay waters in this area. However, a second flow regime is established during the freshet period when the Susquehanna River flow (net non-tidal) is increased by spring flooding. In this case, the head of the bay is flushed as the river flow overpowers the weak tidal circulation and all materials move seaward.

In general, the size of the materials averages between 1.2 and 2.6 microns, with 65 percent lying in the range 1.2 to 1.6 microns. On the basis of water temperatures and salinities characteristic of the upper bay waters, the settling velocity for these particles is less than 10^{-3} cm/second. Natural background turbulence in the shallow waters of the bay generally exceed this velocity; thus, the bulk of the finest particulate matter is maintained in suspension, and in the event it is deposited, it is susceptible to resuspension by tidal currents. Thus the fine fraction of upper bay sediment, which has been shown to act as the vehicle for transport of chlorinated hydrocarbons materials, remains in a state of flux.

Centers of deposition in the turbidity maximum, in the Baltimore harbor area, and in the wide bay floor between the mouth of the harbor and the Chester River show elevated concentrations of chlorinated hydrocarbons. Values are generally lower at the mouth of the Susquehanna River, a result of the coarse nature of the bottom materials in that area. The finer suspended matter carried by the river water bypasses this site, finally coming to rest in the upper Chesapeake Bay either through direct settling from suspension or, more likely, through organic agglomeration (e.g., metabolic byproducts of zooplankton and benthos). There is no clearly defined longitudinal gradient in mean grain size along the axis of the bay; although, the distribution of the background particle population has been found to be similar from year to year.

In light of: (1) the contribution of suspended matter from the Susquehanna River (and lesser amounts from the tributaries to the upper bay) and (2) the fact that concentrations of sediment remain essentially the same, the suspended sediment budget demands that an equivalent amount be lost from the waters of the upper bay. Thus, the flux is thus either downward by sedimentation or seaward by longitudinal transport. Previous work has revealed that by far the greatest amount is lost to the bay floor; thus, the fine fractions bearing the highest concentrations of chlorinated hydrocarbons are accumulating as bottom deposits.

The average concentrations of PCB, chlordane, and DDTR were highest in suspended sediments filtered from the water in Baltimore harbor, with high individual values of 3.8 ppm for PCB, 0.34 ppm for chlordane, and 0.30 ppm for DDTR being recorded. When these data are presented as the amount of CHC present in the water column on suspended sediment, the average CHC values show a decreasing trend southward down the bay with the harbor station not as high in the case of PCB or, about the same in DDTR and chlordane as the upper stations. A positive correlation was found between the concentration of suspended sediments in the water and the concentrations of CHC's in the water column on suspended sediments, a factor which reflects the adsorbtive capacity of the finer fractions of Bay sediments.

——H. D. Palmer

4.3 Marine Biology

In zooplankton larger than 202 microns, standing stocks were highest in the late winter months and minimal in early summer and early fall. The average standing stocks increased from Station 1A down the bay. Baltimore harbor was distinct from the other stations in that the average standing stocks exceeded other bay localities by a factor of four to five. The biomass calculations indicated that the collections from the more northerly stations contained more organic debris than did the other stations.

The species diversity showed a decreasing trend from Station 1A down the bay. Of the 90 different forms identified and counted, the most dominant groups were the copepods (27 species) and the cladocerans (20 species). Oak leaf hairs were found to be the second most abundant form in terms of frequency of occurrence. The largest numbers of oak leaf hairs were collected at the more northern stations.

The community correlation coefficients suggest four basic zooplankton communities in the study area: (1) a basically freshwater community extending from Havre de Grace to Turkey Point, (2) a community in the vicinity of Pooles Island, (3) a distinct community in Baltimore harbor dominated by the copepod *Eurytemora affinis*, and (4) another community extending from below the Patapsco River to about the Severn River.

The CHC residue concentrations found in zooplankton samples varied tremendously in time and space—so much, that the sampling frequency probably was not sufficient (temporally or spatially) for the average values to be entirely representive. The concentrations of PCB and DDTR in the zooplankton were highest at Station 1A at the head of the bay (maximum values of 7.5 ppm PCB and 4.2 ppm for DDTR). The most consistently high chlordane values in zooplankton were found in Baltimore harbor, the highest being 0.14 ppm. When the amount of CHC present in the water column but contained in the zooplankton biomass was calculated, a positive correlation was found with the zooplankton biomass.

The shellfish in the upper bay were found to have about the same levels of PCB, chlordane, and DDTR as the shellfish in the Chester River. The shellfish concentrated the CHC's many thousands of times higher than the levels in the water column (e.g., PCB, 4,000; chlordane, 30,000; and DDTR, 45,000).

Toxaphene was observed in only one biological sample in the upper bay—a zooplankton sample from Baltimore harbor.

The laboratory experiments with the Aroclor® 1254 PCB formulation, demonstrated that PCB is rapidly adsorbed from the water by suspended clay particles. Data from the *in situ* experiment show that when PCB was added to Chesapeake Bay water containing only 10 to 20 mg/l suspended sediment, essentially all of the recovered PCB is contained on the suspended sediment rather than free in the water.

The clams and oysters in the *in situ* experiments were able to take up the PCB from the suspended sediments, in some cases accumulating concentrations 1,000 times higher than the concentration administered. Raising the water temperature increased the PCB uptake dramatically—perhaps by increasing the shellfish pumping rates, thereby increasing the amount of PCB-containing suspended sediments passing through the animals. As in previous experiments with chlordane, the clams accumulated the PCB even at the very low ambient water temperature (4.7°C). The oysters accumulated the PCB very slowly at water temperatures below 10°C. As in the chlordane experiments, from a given level administered, the oysters were able to accumulate higher levels in their tissues than were clams.

——J. M. Forns

4.4 Microbiology

The microbiology of suspended particulates, bottom sediments, and water of the upper Chesapeake Bay was examined. Co-transportation of chlorinated hydrocarbons, bacterial indicator organisms, and potential pathogens via suspended sediment was found to occur. The highest total viable counts (TVC) of bacteria in bottom water samples were observed during the winter and spring months; the lowest were observed in July, September, and October. Total viable counts of bottom sediment samples followed essentially the same distribution. The highest recorded counts over the year, obtained at Station 1A, were ten to a hundred times greater than at Stations 5A and 10B.

Most probable number (MPN) levels of total coliforms, fecal coliforms, and fecal streptococci fluctuated seasonally and spatially from Stations 1A to 11A. In general, MPN values decreased from winter to summer, with a gradual increase in the number of indicator organisms in the fall, except that higher MPN values occurred at Station 11A in June through September. Total coliform levels were higher than fecal coliforms and fecal streptococci levels, with the highest MPN values being found in water samples from Station 1A. The entry of total coliforms to the upper Chesapeake Bay via the Susquehanna River appears to be significant. MPN values at all of the sampling stations were lower in bottom sediments than in the water, with relatively less of a seasonal fluctuation occuring in the sediments. Fecal streptococci levels were generally higher than total and fecal coliform levels in bottom sediment.

Approximately 80 percent of the fecal coliforms were *Escherichia coli*, Type I. Station 10B more commonly gave false positive coliform MPN's. More than 80 percent of the fecal streptococci were entercocci.

More than 33 percent of the FC:FS ratios were greater than 4.0 for Station 1A samples, including suspended sediment samples.

The relative proportions of fecal coliforms and fecal streptococci in the total viable counts decreased southwards from Station 1A to 11A during the winter months, were uniform during the spring months, and rose starting in June at Station 11A.

A highly significant proportion of both total viable bacteria and selected indicators of fecal pollution were found associated with particulate matter in the water column. Up to 53 percent of the total viable bacteria in Station 11A water samples was found to be associated with particulate matter.

By means of a non-selective enrichment, *Salmonella enteritidis* was isolated from samples collected during this study. *Clostridium botulinum* Types B and E, *Vibrio parahaemolyticus,* and *Yersinia* sp. also were isolated.

Approximately one to ten percent of the total viable bacterial population was found to be resistant to and potentially capable of metabolizing PCB 1254. These bacteria were present at all stations.

Greater amounts of humic acid were found in samples collected at Station 1A, and the humic acid concentration decreased from Station 1A to Station 11A.

——R. R. Colwell

4.5 Numerical Modeling

A numerical model has been developed to predict the source field from the concentration field of a passive contaminant subject to advection, diffusion, and vertical settling velocity (Volume IV). The model is three-dimensional and in this case is applied to the upper Chesapeake Bay. However, it can be adapted readily to any other body of water.

The complete model consists of ten discrete programs. The reasons for this segmentation are twofold:

(1) All the programs were designed to be run on computers of relatively low storage capacity (less than 20,000 numbers) such as the IBM 7094 at Johns Hopkins. For a three-dimensional model, this imposes a very strong constraint, and multiple programs are necessary.

(2) Segmentation increases the versatility of the model as a whole. In the future, sections of the model may be modified readily in response to changes in input data quality and quantity, output requirements, and computer capabilities. Furthermore, certain aspects of the present modeling sequence are far from ideal. For example, an intrinsic requirement of a transport process model for a contaminant in a fluid is the velocity field of that fluid. This could be derived: (1) from a great quantity of current meter observations quite beyond the capabilities of the Upper Bay Survey or (2) a three-dimensional dynamic model of the water motions in the upper bay. Such a model does not exist at present; hence, a pseudo-dynamic model was developed that predicts in a grossly simplified manner a plausible velocity field, based on observations of currents at the boundaries. This section of the total model, no doubt, can be replaced when present two and three-dimensional dynamic estuarine models have been adequately verified and developed into useable tools. (Examples of such models are those of Leendertze et al., 1973, and Caponi, 1973).

The best estimates of sources and sinks so far show the fluxes of sediment through the northern and southern open boundaries. The variations in source field across the southern boundary in the low flow case may be due to errors in the input data in this region or an artifact of the model. An interesting and probably real feature of the source fields, especially in the southern and middle areas of the model, is the way in which many regions are shown as a source in one season and a partially-compensating sink in the other. The source field thus shows a strong variation from season to season, many regions alternating between sedimentation and resuspension during the year.

It is important to note that some regions of the model have little or no suspended sediment data; hence, the predicted source fields in these regions are a function of the interpolation procedures used rather than of any real phenomena. Such regions are the Chester River and the mouths of the Gunpowder, Middle, and Back Rivers.

It must be emphasized that the vertically integrated sediment source fields derived here are only tentative. They are a function of: (1) the velocity field, (2) the suspended sediment concentration field and the interpolation procedure used to derive it, and (3) the fact that horizontal diffusive exchange is neglected. Ideally, investigations should be carried out as to the sensitivity of the predicted source field to these input parameters and to the factors upon which they depend.

Based on the availability of current velocity and suspended sediment data at the present time, it would appear that the most reasonable predictions of suspended sediment sources can be obtained by the following combination of models:

(1) A pseudo-dynamic model of the velocity field in three dimensions or, better, a full dynamic model (which does not at present exist).

(2) A prediction model for the vertically integrated source field of suspended sediment, based initially on a zero horizontal exchange coefficient and later on a finite one if it appears necessary.

Data requirements for one run of the model are:

(1) The discharge of the Susquehanna River at Conowingo Dam.
(2) Current meter records at the southern open boundary of the model (roughly three moorings with three meters per mooring—these data to be used as the boundary condition for the pseudo-dynamic model) and in the interior of the model (roughly three moorings with three meters per mooring—these data to be used as a means of choosing the optimum value of K in the pseudo-dynamic model). The duration of these records should be at least 30 days and the recording interval 30 minutes or less.
(3) Vertical profiles of the suspended sediment concentration (and, if applicable, the concentration of sediment-borne contaminant) at roughly 30 stations in the Upper Bay.

——J. D. Hunter

4.6 Data Base

The Upper Bay Survey Data Base system was a useful and efficient tool for managing, analyzing, and presenting the many kinds of data collected during the survey, the laboratory analyses, the report writing, and the culmination of the project (Volume III). It met with a high degree of success in achieving the objectives as stated in the proposal and incorporated in the contract. These objectives for the project's automated data processing operations were to:

- Plan, design, and operate an automated data handling system.
- Coordinate and conduct the collection and assembly of all data to be stored and/or processed by computers.
- Provide for storage and rapid retrieval of data for study participants.
- Provide data manipulation, statistical analyses and correlation capability for study participants.

The data base's management feature facilitates the quick retrieval of any portion of the data and provides an inexpensive method of storing these data when not in use. The data base is easily updated with new data and expanded to provide for new data parameters. In the course of the project, it was demonstrated that it was possible to enter new data items into the system with ease as soon as they become available and to retrieve any part of the archived data for examination, comparison, or analysis.

It was particularly gratifying to demonstrate that these things can be accomplished regularly by the principal investigators and, in many cases, by their assistants and clerical personnel after a minimal period of instruction.

Special data manipulation subroutines were established and stored in the system for report writing, simple statistical calculations, and some elementary multidisciplinary correlations. The success of these demonstrated the capability of the system to produce answers to special questions or to prepare special data tables and reports in response to particular needs and requirements of public agencies and officials concerned with natural resources management. The flexibility of the Upper Bay Survey Data Base in creating data reports in response to *ad hoc* requests for information from various users makes the system highly suited for a wide scope of applications.

The limitations experienced were typical to electronic data processing systems. The lag time between a sampling or measurement event and entry of the values into the system makes concurrent analysis very difficult. Errors in formatting, incorrect data, key punch errors, and procedural errors were all experienced, but they were overcome with a reasonable amount of persistence.

——A. R. Barskis

CHAPTER 2

MARINE BIOLOGY

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ABSTRACT

A one-year field sampling and experimentation program was undertaken in the upper Chesapeake Bay as part of an interdisciplinary evaluation of the mechanisms, rates, and routes of chlorinated hydrocarbon transport through aquatic biota. The biota investigated included the fraction of the zooplankton community greater than 200μ and the sessile benthic shellfish including clams (*Mya arenaria*) oysters (*Crassostrea virginica*), and brackish water clams *(Rangia cuneata).*

Field collections were made nine times during one calendar year from approximately 18 stations distributed throughout the upper Chesapeake Bay from Annapolis to Harve de Grace. Biomass, taxonomic, and biochemical evaluations were performed on the resulting samples. Quantifications were made of the abundance, distribution, and chlorinated hydrocarbon content for the zooplankton. Chlorophyll-a was measured; physical parameter profiles of temperature, salinity, pH, and dissolved oxygen also were taken.

Experiments were performed in the laboratory to identify possible mechanisms by which quantitated fractions of chlorinated hydrocarbons became available to aquatic biota, particularly commercially-sought shellfish. Combining the results of the interdisciplinary field sampling survey with the initial laboratory tests, a series of *in situ* shellfish experiments were conducted. Clams and oysters were subjected to chlorinated hydrocarbon (Aroclor® 1254) stresses under realistic conditions at various temperatures, and accumulations within whole body tissue were measured.

Results of this program indicate that zooplankton communities are controlled and distributed throughout the upper bay by the estuarine mixing characteristics regulated by temperature and salinity regimes. Although distinctly different communities of zooplankton were observed, organism diversity or abundance was not related to sources or levels of chlorinated hydrocarbons in the aquatic environment.

Chlorinated hydrocarbon accumulations by shellfish are regulated to a great extent by their pumping rate which, in turn, is dependent on the environmental parameters. Oysters appear to remove chlorinated hydrocarbons effectively from non-viable suspended sediments at ambient temperatures above 10°C. Clams, however, seem to accumulate chlorinated hydrocarbons irrespective of temperature, and their total body burdens of chlorinated hydrocarbons fluctuate. Comparison of oysters and clams from the same experiment indicates that each species behaves differently, and projections of biological effects based solely on source water characteristics cannot be made.

TABLE OF CONTENTS

Section		Page
2.	MARINE BIOLOGY	2-1
2.1	Introduction	2-1
2.2	Plankton Investigations	2-2
2.2.1	Sampling and Analytical Methodology	2-4
2.2.2	Results and Discussion	2-5
2.3	Benthic Investigations	2-15
2.3.1	Field Investigations	2-20
2.3.2	Laboratory Experiments	2-20
2.3.3	*In Situ* Experiments	2-23
2.4	Conclusions	2-34
2.5	Acknowledgements	2-35
2.6	References	2-35

LIST OF FIGURES

Figure		Page
2-1	Net Plankton Sampling Station in the Upper Chesapeake Bay, December 1973 through December 1974	2-3
2-2	>202μ Net Plankton Standing Stock, Annual Average Concentrations by Month	2-10
2-3	>202μ Net Plankton Average Annual Standing Stock by Station	2-11
2-4	Frequency Distributions of Biomass Values for Upper Chesapeake Bay Zooplankton	2-12
2-5	Biomass Ratios of Average Monthly Data	2-13
2-6	Biomass Ratios for Principal Stations Sampled Throughout the Upper Chesapeake Bay	2-14
2-7	202μ Net Plankton Investigations Taxonomic Data Analysis: Community Correlations	2-19
2-8	Low Percentage Organic Clay Adsorption with PCB 1254	2-22
2-9	*In Situ* Experimental Laboratory System	2-24
2-10A	Laboratory Module on *R/V SEARCHSTAR* Being Towed to Station	2-25
2-10B	High Pressure Particle Separator	2-25
2-10C	Organism Test Chambers Having a Two-Liter per Minute Flow Rate	2-25
2-11	Regressions on Biomass Animal Sorting Evaluations	2-28
2-12	PCB-1254 Uptake by Oysters During *In Situ* Test	2-32
2-13	PCB Uptake by Clams During *In Situ* Test	2-33

LIST OF TABLES

Table		Page
2-1	Plankton Biomass for the Upper Chesapeake Bay: A Sample of the Data	2-6
2-2	Plankton Taxa from the Upper Chesapeake Bay: A Sample of the Data	2-7
2-3	Zooplankton Biomass Annual Average Concentrations by Month at all Stations	2-8
2-4	Zooplankton Biomass Annual Average Concentrations by Station	2-9
2-5	Biomass Ratios Based on Monthly Average Concentrations at All Stations	2-9
2-6	Dominant Forms from Upper Chesapeake Bay	2-16
2-7	>202μ Net Plankton Species Diversity by Station for Upper Bay	2-17
2-8	>202μ Net Plankton Species Diversity by Station for Upper Bay	2-17
2-9	Average Number of Species per Station from Upper Chesapeake Bay	2-18
2-10	Results of Low Percentage Organic Clay Absorption Experiment	2-21
2-11	PCB 1254 Absorbed to Suspended Sediments During *In Situ* Experiment	2-26
2-12	Oyster Biomass Sorting Evaluations for *In Situ* Experimentations	2-26
2-13	Clam Biomass Sorting Evaluations for *In Situ* Experimentations	2-27
2-14	Physical Parameter Measurements During *In Situ* Dose Experiment, Station 11A	2-30
2-15	Data from *In Situ* Experiment at Station 11A	2-31

2. MARINE BIOLOGY

2.1 Introduction

The aquatic biology task of the Upper Bay Survey was planned to include essentially two estuarine communities of organisms, the zooplankton and the benthos. With respect to the overall objectives of the Upper Bay Survey to determine rates, routes, sources, sinks of chlorinated hydrocarbons (CHC) — the organisms investigated were to provide a means of understanding the areal distribution and abundances of chlorinated hydrocarbons, potential localized sources of these compounds to the waterways, and initial identification of potential biological transfer mechanisms for these nondegrading chemicals. Consideration of these objectives led to selecting for investigation the fraction of the plankton community retained in a 202-micron mesh sampling net and the sessile benthic community.

The selection of this $>$ 202-μ fraction of the zooplankton was based on several factors. From the recent literature, there seems to be reasonably adequate knowledge of the abundance, distribution, and productivity of the smaller fraction ($<$ 202μ) zooplankton and phytoplankton populations. Also, it was felt that there was a definite lack of detail for the larger zooplankton, especially such meroplanktonic forms as fish eggs and larvae. The fact that no definitive effort was planned in this program to address fish populations also influenced the decision to choose the $>$ 202-μ fraction of zooplankton. Because it would be quite difficult to document the source of chlorinated hydrocarbons measured in relatively transitory fish stocks, the obvious alternative was to investigate from upper bay waters the potential food sources which might contain these chemicals.

Selection of the molluscan fraction of the benthos from the upper Chesapeake Bay was based on several considerations. It was felt that these organisms, which are primarily sedentary filter feeders, would provide a means of utilizing stationary benthic populations as biological samplers of chlorinated hydrocarbons. From previous studies in the Chester River, it was believed that a principal mechanism for CHC availability comes from the suspensoid material within the water column. More definitively, the inorganic suspended sediments act as a site for adsorption of these insoluble chemical compounds which can then be passed through the benthic organisms. During certain times of the year when organic production is at a minimum, the filter feeding molluscs actually can ingest detritus as part of their diet, especially when winter water temperatures are around or above 10°C.

Another reason for selecting the molluscs for this investigation was their ubiquity throughout the upper bay. From previous assessments by the Maryland Department of Natural Resources, the brackish water clam, *Rangia cuneata*, was distributed throughout the upper Chesapeake Bay except in Baltimore harbor.

Finally, utilizing the molluscs as investigative organisms provided a means of making direct assessments of potential environmental impacts to commercially important species in Chesapeake Bay. By working with oysters (*Crassostrea virginica*) and soft-shelled clams (*Mya arenaria*), it was felt that a valuable insight could be gained with respect to the management of these renewable natural resources.

The survey program was divided into two discrete phases: the first to include a one-year field sampling effort throughout the upper bay and the second to involve experimental investigations with chlorinated hydrocarbons and shellfish. Conducting field sampling in conjunction with the other scientific disciplines gave insight to the background levels and sources of CHC's in plankton and benthos. From this baseline of data, experimental designs were established, and low level toxicity testing was conducted.

The results of evaluations in both phases of the marine biological task led to definite conclusions. While the conclusions are by no means complete, they do provide a means of understanding the levels and concentrations of CHC in zooplankton and benthic organisms. With reasonable interpretation, the resultant information can be used to forecast chlorinated hydrocarbon conditions in plankton and benthos of the upper Chesapeake Bay.

2.2 Plankton Investigations

Zooplankton populations throughout the upper Chesapeake Bay were examined during December 1973 through December 1974. During that time, ten series of samplings were made from the *R/V MAURY, R/V WARFIELD* or *R/V NORTHSTAR.* Eighteen stations were visited, resulting in 95 paired net plankton samples (Figure 2-1). Volume III contains a complete inventory of the data described here. The objectives for which the samples were collected were to:

(1) Determine the seasonal biomass abundances for the > 202-μ net plankton fraction of the upper Chesapeake Bay.

(2) Identify and quantitate the species distribution for these upper bay fauna.

(3) Analyze for background levels of chlorinated hydrocarbons within these organisms.

(4) Compare the resultant plankton data with chlorinated hydrocarbon levels in water, suspended sediments, and bottom sediments.

The rationale for this program was based on several premises gained from reported literature in similar estuarine waters and from initial observations made during the Chester River Study in 1972. Plankton serve as biological samplers of chlorinated hydrocarbons within the water column and, since these organisms rely on hydrographic whims for their mass transport and distribution, their movements may serve as a means of detecting CHC trajectories, distribution, and possible source areas. Also, this > 202-μ fraction of the plankton community serves as an intermediate level of the aquatic food web and may indicate a role in bioaccumulation processes. Finally, it has been well documented that the more sensitive stages of many aquatic organisms occur during their life cycle when they lead a meroplanktonic existence and are within the > 202-μ fraction of water column biota.

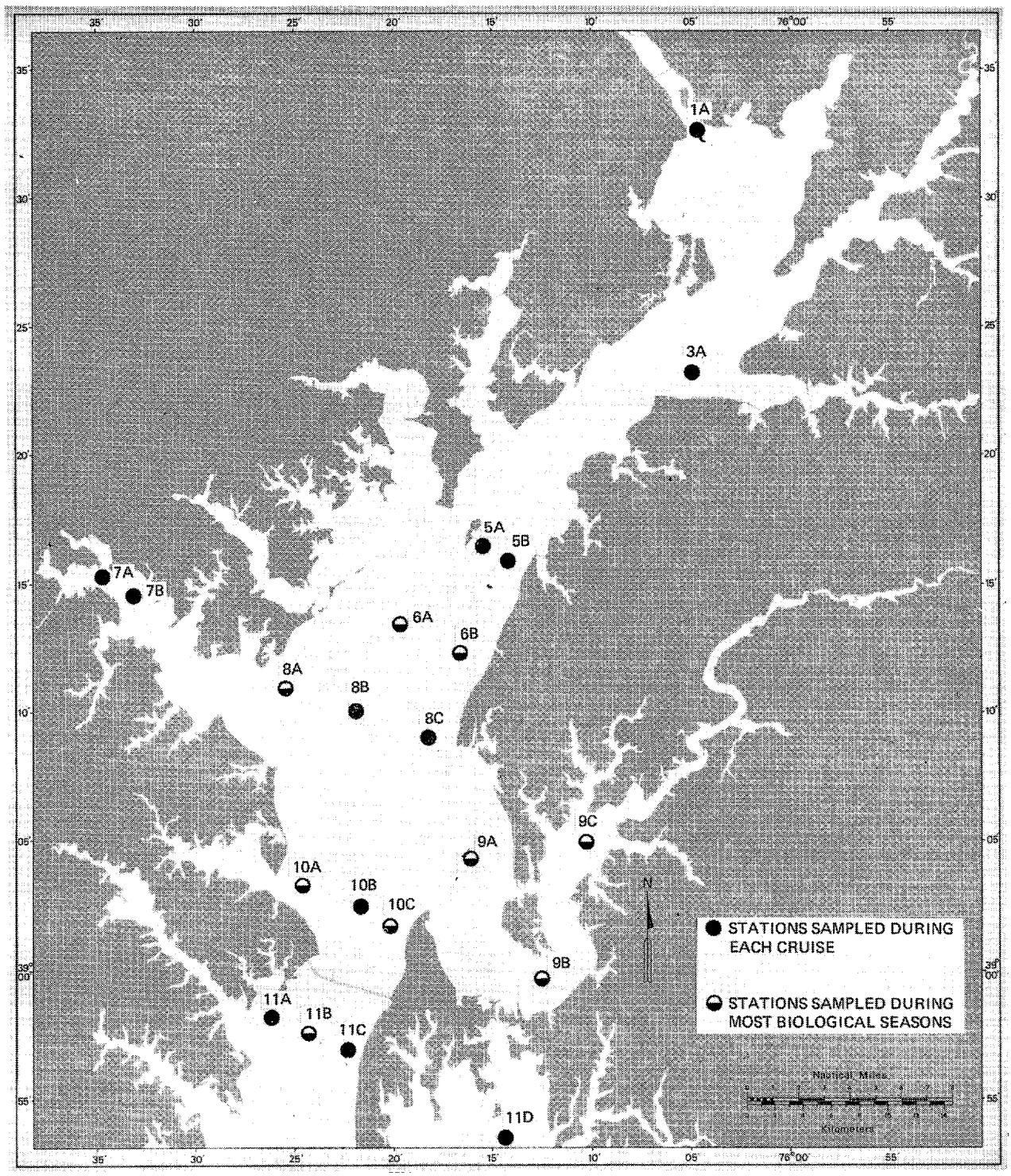

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Figure 2-1. Net Plankton Sampling Stations in the Upper Chesapeake Bay, December 1973 Through December 1974

2.2.1 Sampling and Analytical Methodology

The collection of these plankton samples was made with paired half-meter standard oceanographic nets using hexane-rinsed 202-μ Nitex netting. A calibrated T.S.K.-type mechanical flowmeter was placed in the mouth of each of the two nets to record the volume of water passing through. A third was placed equidistant above the nets to record the unobstructed water flow during towing. From the timed flow values measured by the three flowmeters, were calculated the volumes of water filtered in each net, net filtering rate, and a measure of each net's filtration efficiency. The reduced calculations from the 95 paired samples showed that the average filtration rate was 14.6 m^3 per minute at an average towing speed of 1.4 meters/second ($\pm$0.6). The average net filtration efficiency for the 95 collections was 73 percent with the lower efficiencies occurring during the June, July, and September cruises.

Each net haul consisted of towing obliquely from surface to near-bottom to surface for 2.5 to 5 minutes. Upon completion of each tow, the nets were carefully washed to concentrate the plankton in the cod end. The cod end was then removed, and the sample was washed through a 4-mm PVC-stainless steel sieve to separate out the larger gelatinous material. The large organism fraction, consisting mostly of medusae and comb jellies, was counted; the volumes were recorded, and the gelatinous forms were discarded. All large non-gelatinous forms were recorded and placed in the sample fraction which passed through the 4-mm sieve. The samples were then concentrated through a 202-μ sieve and brought to a volume of approximately one pint. From each pair of net samples, the net collection for taxonomy and biomass was preserved with buffered formaldehyde at a final concentration of five percent. The net collection used for biochemical analysis was further concentrated on hexane-extracted 47-mm Gelman glass filters. The resultant sample pad was wrapped in aluminum foil and frozen for laboratory analysis ashore. During the summer cruises, several individual parts of samples were removed, and individual species were isolated for micro-extraction and biochemical analysis. A total of 12 micro-samples were isolated for individual analysis. Unfortunately, this particular effort proved to be fruitless — the first few sets of samples were found to contain insufficient biological material to yield chlorinated hydrocarbon values above the detection limits, and adequate time was not available for analysis of the larger samples isolated later.

Profiles of physical parameters (including temperature, salinity, dissolved oxygen, and pH) were collected at each sampling station. Total seston mass was measured on millipore filters, and chlorophyll-a extractions in acetone also were accomplished for each plankton sampling station.

The frozen samples were thawed in the laboratory, and prepared for chemical extractions according to the procedures described in Section 6. The formalin-preserved samples were washed with distilled water and split in a modified Folsom splitter to a manageable aliquot not less than one thirty-second of the whole sample. The working aliquots were kept in distilled water, and the remaining sample was transferred sequentially to a 10 percent ethyl alcohol solution for final preservation and archiving. Experience shows that initial buffered-formalin fixing followed by alcohol preservation is the least deleterious to the samples, and this procedure reduces the amount of solution which takes place when organic acids reduce pH in buffered-formalin preserved samples. Also, it appears that natural pigments are better retained by this method.

The taxonomic analysis of the net plankton collections consisted of placing the split aliquot in a graduate, bringing the sub-sample to 100 ml and removing 10 ml by Stemple pipet. This fraction was then placed in a gridded sorting tray, and two series of identification and counts were undertaken. Initially, a 10 percent count was made for the dominating forms, followed by a 100 percent count for all other organisms. Efforts were made to identify the dominating organisms to the species level and less abundant forms at least to the group level. Normally, by this method about 500 to 2,000 organisms were examined from each sample aliquot, and counting reproducibility was greater than 75 percent. All data were transferred then to the initial format sheets for numerical computation to reduce the tray counts to numbers of organisms per cubic meter and relative percent composition. These data then were transferred to the standardized formats for entry into the data base (Volume III).

Five measures of biomass were made on the 95 samples analyzed: wet weight, displacement volume, dry weight, ash weight, and ash-free dry weight. From experience with previous estuarine investigations, it was found necessary to perform these analyses to discriminate the truly planktonic organisms from the sometimes substantial quantities of debris collected in the nets. Detailed procedures described in the Chester River Study (1972) and Forns (1973) were used for the biomass evaluations. Wet weights were determined by (1) washing the sample matter into a precalibrated, fritted glass crucible, (2) applying slight pressure to force out interstitial water, and (3) weighing on a Mettler H20 analytical balance. Displacement volumes were measured by the Gooch crucible mercury immersion method described by Yentsch and Hebard (1957). Dry weights were determined by the procedures of Lovegrove (1966), and ash weights were determined by furnace combustion to 500°C over a two-hour period. The data from these analyses, chlorophyll-a, and seston measurements can be found in Volume III, Section 4.

2.2.2 Results and Discussion

Biomass analysis has been completed for all 95 samples. Taxonomic evaluations have been accomplished for 51 samples, and approximately 70 chlorinated hydrocarbon analyses have been completed on the frozen plankton samples. The resultant biomass data were expressed as microliters per cubic meter ($\mu l/m^3$) for displacement volumes and as milligrams per cubic meter for all other measurements (Table 2-1). The taxonomic data have been expressed as numbers of organisms per cubic meter and as relative percent composition in each sample (Table 2-2). The species distributions for the taxa have been computed according to Margalef (1951) and Shannon and Weaver (1963) for each sample, and community correlation coefficients have been calculated according to Jaccard (1961). The chlorinated hydrocarbon data have been presented first as micrograms (10^{-6} grams) of CHC found divided by the grams wet weight of zooplankton extracted (parts per million, ppm), and additionally as nanograms (10^{-9} grams) of CHC found in the sample divided by the liters of bay water filtered to obtain the sample (parts per trillion, ppt). These varied means of expressing biomass standing stock, taxonomic composition, and CHC data make this material compatible with similar investigations conducted at Woods Hole (Harvey, 1973), the Southern California (SCWRRP) area (Young, 1975) and other estuarine studies.

Net zooplankton biomass data have been reduced in Tables 2-3 and 2-4 to show: (1) annual average concentrations expressed by month throughout the entire upper bay and (2) annual average biomass concentrations displayed by station for the principle stations sampled throughout the year. Graphically, Figure 2-2 indicates that highest overall net plankton standing stock occurs during the late winter months with seasonal low concentrations in early summer and early fall. This slightly bimodal distribution coincides well with productivity values when higher biomass and primary production blooms occur prior to the increases in secondary trophic levels (which comprise the bulk of the - $>$ 200-μ plankton fraction). Comparison of the biomass values from Table 2-4 and Figure 2-3 indicates increasing average plankton standing stocks from the mouth of the Susquehanna River (Station 1A) down the bay to Annapolis (Stations 11A and 11C). The obvious exception lies in the samples from the Baltimore harbor area (Stations 7A and 7B) where biomasses were generally four to five times greater than any other station. This occurrence is also seen in Figure 2-4 where the frequency distribution of biomasses are skewed to the left toward lower values, except for those from Stations 7A and 7B. Opposite sampling stations across the bay (Stations 5A vs 5B, 8B vs 8C, and 11A vs 11C) reveal higher average concentrations on the eastern side of the bay. While these values coincide quite well with the STD profiles taken at the plankton sampling stations (Volume III), it seems somewhat contrary to the classical axiom of higher productivity in the shallower waters of the estuary.

Biomass ratios from monthly average concentrations (Table 2-5) were computed in an effort to differentiate the non-viable detrital material ($>$202 μ) from the actual planktonic organisms. From the ten monthly observations, the biomass ratios are found generally similar to those reported previously (Forns, 1975). However, comparison of the dry weight to wet weight ratios and of the ash-free dry weight to wet weight ratios indicate unusually high values for the October series of samples (Figure 2-5). Comparison of these ratios from Table 2-4 on a station basis indicates very little change in biomass ratios for the 11 principal stations sampled throughout the upper bay (Figure 2-6). The somewhat higher values found at Station 1A are due undoubtably to the usually high amounts of debris collected in the nets from the Susquehanna River.

TABLE 2-1. PLANKTON BIOMASS FOR THE UPPER CHESAPEAKE BAY FROM DECEMBER 1973 TO DECEMBER 1974: A SAMPLE OF THE DATA, CONTAINED IN VOLUME III.

BIOMASS DATA
75/07/18.

SAMPLE NUMBER C703	STAT ION C702	DATE MM DD YY C705	TIME HRS C706	BIOMAS MG M3 C713	BIOM WET MG M3 C714	BIOM DRY MG M3 C715	BIO- ASH MG M3 C716	ASH-FR D MG M3 C717
1	11A	12/12/1973	1100	158.29	128.34	17.135	15.879	1.236
2	10B	12/12/1973	1300	133.81	99.59	13.094	11.756	1.338
3	05A	12/13/1973	1025	110.66	80.32	9.501	.000	.000
4	01A	12/13/1973	1410	126.98	50.98	20.499	5.986	14.513
5	09A	12/14/1973	1110	243.06	115.14	16.528	15.417	1.111
6	08A	12/14/1973	1350	173.29	65.42	89.531	82.455	7.081
7	07A	12/14/1973	1515	999.99	990.99	99.999	99.999	21.471
8	09A	01/17/1974	928	121.56	118.06	15.651	13.751	1.809
9	09B	01/17/1974	1037	491.68	434.73	66.786	61.050	5.736
10	09C	01/17/1974	1132	109.16	248.42	41.637	37.739	3.899
11	11A	01/23/1974	842	120.22	121.02	15.009	13.260	1.749
12	11C	01/23/1974	1000	200.58	164.47	23.607	20.675	2.932
13	07A	01/23/1974	1305	467.11	476.93	59.601	53.806	5.795
14	10B	01/23/1974	1635	69.26	59.70	8.797	7.273	1.524
15	08B	01/24/1974	925	36.23	39.41	5.631	11.094	-5.463
16	05A	01/24/1974	1100	14.34	11.89	1.706	1.276	.430
17	01A	01/24/1974	1400	123.84	137.09	47.183	17.833	29.350
18	03B	01/24/1974	1610	38.43	33.97	7.480	4.228	3.252
19	05B	01/24/1974	1655	8.09	7.91	.956	.706	.250
20	08C	01/24/1974	1745	26.32	21.28	2.807	1.028	1.780
21	07A	03/19/1974	1105	877.91	838.90	97.546	90.474	7.072
22	11D	03/19/1974	1413	999.99	999.99	99.999	99.999	-2.138
23	11A	03/20/1974	820	479.93	492.85	67.559	60.545	7.014
24	11C	03/20/1974	945	999.99	999.99	99.999	99.999	8.901
25	10B	03/20/1974	1110	290.49	284.39	38.635	37.473	1.162
26	08C	03/20/1974	1200	899.76	999.99	99.999	99.999	13.301
27	08B	03/20/1974	1215	371.88	349.33	40.442	33.469	6.973
28	05A	03/21/1974	845	97.56	84.93	9.073	7.659	1.415
29	01A	03/21/1974	1310	18.30	21.13	2.745	1.372	1.372
30	03B	03/21/1974	1430	33.83	29.21	6.653	5.300	1.353
31	05B	03/21/1974	1515	433.18	365.05	40.955	34.851	6.104
32	07B	05/01/1974	850	97.72	55.70	8.404	5.537	2.867
33	11D	05/01/1974	1340	198.90	86.41	12.155	10.387	1.768
34	11C	05/02/1974	755	18.52	9.11	1.148	.889	.259
35	11A	05/02/1974	830	25.71	7.56	.771	.000	
36	10B	05/02/1974	954	6.05	1.60	.182	.000	
37	08C	05/02/1974	1110	6.70	.80	.067	.045	.022
38	08B	05/02/1974	1152	9.02	1.92	.195	.165	.030

TABLE 2-2. PLANKTON TAXA FROM THE UPPER CHESAPEAKE BAY SAMPLED FROM DECEMBER 1973 TO DECEMBER 1974: A SAMPLE OF THE DATA, CONTAINED IN VOLUME III.

MARINE BIOLOGY DATA REPORT PART-B
75/04/16.

SAMP NUMB C703	STAT ION C702	DATE MM DD YY C705	TOTAL-LIP MG**M3 C718	CHL A UGL C719	TOXONOMY ORGANIZM TYPE C726	GENUS SPECIES C727	POPULAT. NUMBER C728	RELT PCMP C729	SEQ NUMB C730
					BRYOZOA	BRYOZOAN STATOBLAST	.05	.00	17
					PLANT	GLOBULAR SEED	.05	.00	18
					PLANT	3 SIDED SEED	.05	.00	19
					PLANT	OAK LEAF HAIRS	.15	.01	20
29	01A	03/21/1974		6.80	COPEPOD	ACARTIA TONSA	.37	.49	1
					COPEPOD	EURYTEMORA AFFINIS	60.38	80.78	2
					COPEPOD	CYCLOPOID A	2.20	2.94	3
					COPEPOD	HALICYCLOPS B	1.65	2.20	4
					COPEPOD	CYCLOPOID C	.09	.12	5
					COPEPOD	EUCYCLOPS	.73	.98	6
					COPEPOD	IMMATURE CYCLOPOIDS	2.56	3.42	7
					COPEPOD	CALANOID NAUPLII	.09	.12	8
					CLADOCERA	BOSMINA LONGIROSTRIS	3.57	4.78	9
					CLADOCERA	DAPHNIA	.09	.12	10
					ANNELID	ANNELID	1.10	1.47	11
					INSECT	INSECT	.09	.12	12
					OSTRACOD	OSTRACOD	.27	.36	13
					TARDIGRADE	TARDIGRADE	.09	.12	14
					ROTIFER	ROTIFER	.46	.62	15
					PLANT	OAK LEAF HAIRS	1.01	1.35	16
					DEBRIS	ORGANIC			17
30	03B	03/21/1974		6.10					
31	05B	03/21/1974		12.60					
32	07B	05/01/1974			COPEPOD	ACARTIA TONSA	.72	.02	1
					COPEPOD	EURYTEMORA AFFINIS	1893.16	44.42	2
					COPEPOD	CYCLOPOID A	.26	.01	3
					COPEPOD	HALICYCLOPS B	2.02	.05	4
					COPEPOD	CYCLOPOID NAUPLII	17.46	.41	5
					COPEPOD	HARPACTICOID A	.39	.01	6
					COPEPOD	HARPACTICOID D	.07	.00	7
					COPEPOD	ERGASILUS CHAUTAUQNS	.26	.01	8
					COPEPOD	CALANOID NAUPLII	.13	.00	9
					CLADOCERA	BOSMINA LONGIROSTRIS	.07	.00	10
					ANNELID	ANNELID	.07	.00	11
					BRYOZOA	BRYOZOAN FLOATOBLAST	1.04	.02	12
					INSECT	INSECT	.13	.00	13
					OSTRACOD	PRIONOCYPRIS LONGFMA	.07	.00	14
					ROTIFER	ROTIFER	.59	.01	15
					PLANT	OAK LEAF HAIRS	2345.28	55.03	16
33	11D	05/01/1974		4.41					
34	11C	05/02/1974		22.30					
35	11A	05/02/1974			COPEPOD	ACARTIA TONSA	56.56	8.28	1
					COPEPOD	EURYTEMORA AFFINIS	34.45	5.04	2
					COPEPOD	IMMATURE CYCLOPOIDS	.46	.07	3
					CLADOCERA	BOSMINA LONGIROSTRIS	.10	.02	4
					CLADOCERA	DAPHNIA	.20	.03	5
					CLADOCERA	PODON	35.99	5.27	6
					BARNACLE	NAUPLII	22.26	3.26	7
					CRUSTACEAN	UNKNOWN LARVAE	.05	.01	8
					OSTRACOD	OSTRACOD	2.42	.35	9

TABLE 2-3. >202μ NET ZOOPLANKTON BIOMASS ANNUAL AVERAGE CONCENTRATIONS IN THE UPPER CHESAPEAKE BAY BY MONTH AT ALL STATIONS

Date	Displacement Volume ($\mu l/m^3$)	Wet Weight (mg/m^3)	Dry Weight (mg/m^3)	Ash Weight (mg/m^3)	Ash-Free Dry Weight (mg/m^3)
12/73	278.011 (±321.296)	218.683 (±341.634)	38.041 (±39.018)	33.070 (±40.429)	6.679 (± 8.308)
1/74	140.525 (±160.336)	144.222 (±155.221)	22.835 (±23.091)	18.748 (±40.030)	4.080 (± 8.102)
3/74	500.256 (±384.585)	496.887 (±396.914)	54.873 (±39.871)	51.922 (±40.030)	4.775 (± 4.495)
5/75	45.005 (± 57.586)	25.013 (± 29.329)	3.340 (± 4.105)	2.512 (± 3.471)	0.906 (± 0.926)
6/74	20.888 (± 16.753)	12.696 (± 17.016)	1.397 (± 1.986)	1.349 (± 1.929)	0.158 (± 0.130)
7/74	41.252 (± 45.147)	34.269 (41.664)	4.150 (± 4.630)	3.742 (± 4.158)	0.409 (± 0.514)
9/74	110.137 (±167.727)	88.758 (±138.086)	10.041 (±14.619)	9.204 (±13.140)	0.837 (± 1.567)
10/74	10.410 (± 6.644)	7.175 (± 3.875)	3.899 (± 1.734)	0.423 (± 0.388)	3.476 (± 1.569)
11/74	127.462 (±149.521)	99.338 (±127.704)	24.291 (±26.151)	20.928 (±32.439)	12.706 (±12.859)
12/74	80.026 (± 81.916)	68.119 (± 70.541)	16.000 (±13.205)	8.120 (± 7.788)	7.788 (± 5.605)

TABLE 2-4. >202μ NET ZOOPLANKTON BIOMASS ANNUAL AVERAGE CONCENTRATIONS IN THE UPPER CHESAPEAKE BAY BY STATION

Station Numbers	Displacement Volume ($\mu l/m^3$)	Wet Weight (mg/m^3)	Dry Weight (mg/m^3)	Ash Weight (mg/m^3)	Ash-Free Dry Weight (mg/m^3)
1A	50.501 (± 45.443)	43.862 (± 41.392)	11.247 (±15.841)	4.620 (± 5.617)	6.626 (±10.431)
3B	48.529 (± 65.798)	38.819 (± 49.597)	6.596 (± 6.312)	4.978 (± 6.411)	1.618 (± 1.564)
5A&B	63.661 (±107.285)	50.479 (± 90.941)	6.969 (±10.203)	4.499 (± 8.710)	2.149 (± 2.701)
7A&B	320.316 (±410.949)	304.411 (±410.029)	34.826 (±43.905)	32.693 (±42.449)	4.818 (± 7.248)
8A&C	129.388 (±243.594)	117.823 (±269.631)	19.966 (±33.887)	23.911 (±38.971)	2.460 (± 4.799)
10B	68.806 (± 87.462)	58.848 (± 85.042)	9.478 (±11.883)	7.069 (±11.378)	2.656 (± 3.511)
11A&C	142.722 (±237.750)	133.305 (±239.521)	18.332 (±25.871)	14.833 (±25.009)	4.391 (± 4.932)

TABLE 2-5. UPPER BAY BIOMASS RATIOS BASED ON MONTHLY AVERAGE CONCENTRATIONS AT ALL STATIONS SAMPLED

Date	Wet/ Displacement Volume	Dry/Wet	Ash/Wet	Ash-Free/ Wet	Ash-Free Dry/ Dry
12/73	78.66	17.39	15.12	3.05	17.55
1/74	102.63	15.83	12.99	2.83	17.86
3/74	99.32	11.04	10.44	0.96	8.70
5/74	55.57	13.35	10.04	3.62	27.12
6/74	60.78	11.00	10.63	1.24	1.13
7/74	83.07	12.11	10.92	1.19	9.85
9/74	80.58	11.31	10.36	0.94	8.33
10/74	68.92	54.34	5.89	48.44	89.15
11/74	77.93	24.45	21.06	12.79	52.30
12/74	85.12	23.48	11.92	11.43	48.67

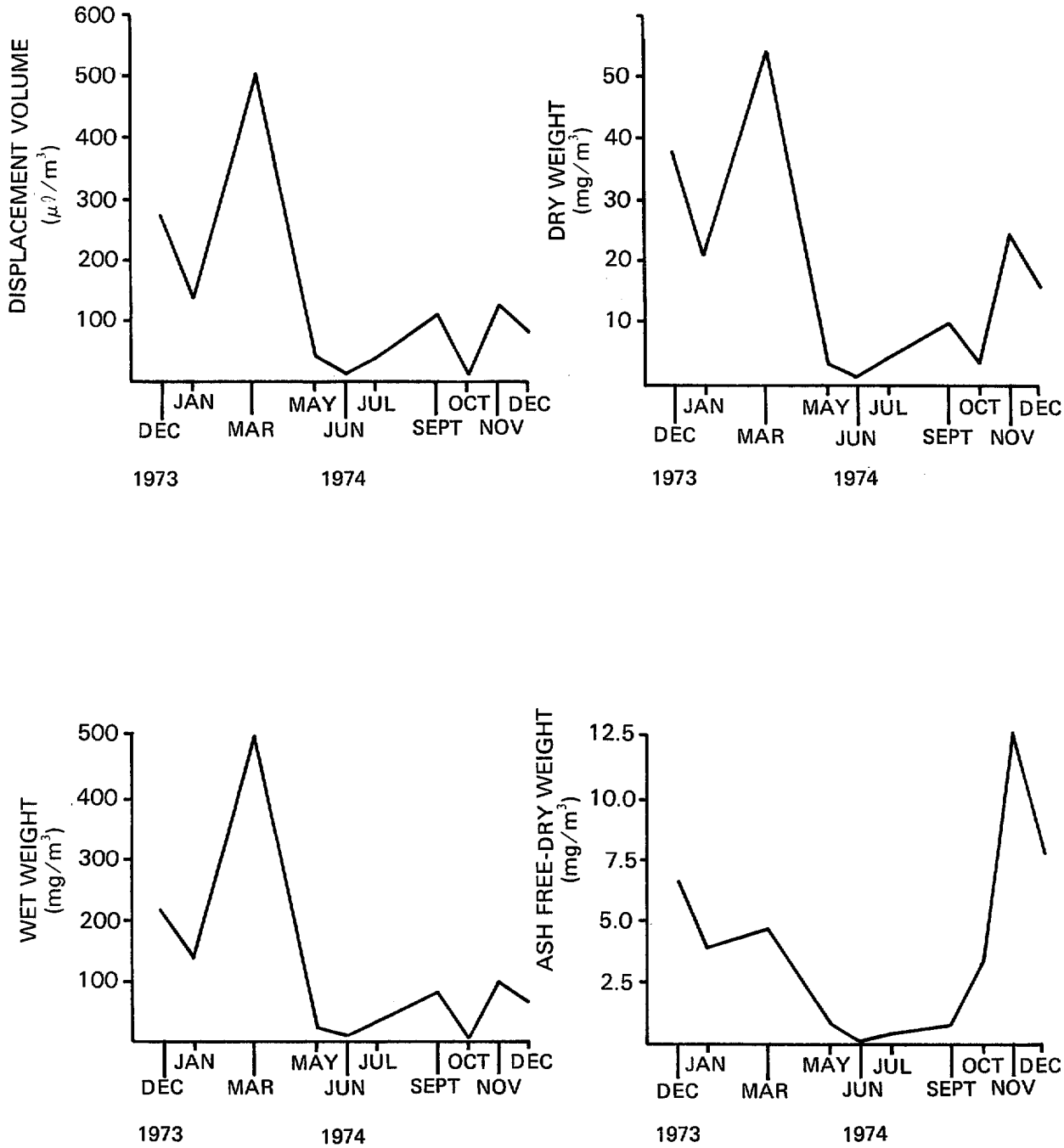

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Figure 2-2. >202μ Net Plankton Standing Stock Annual Average Concentrations By Month

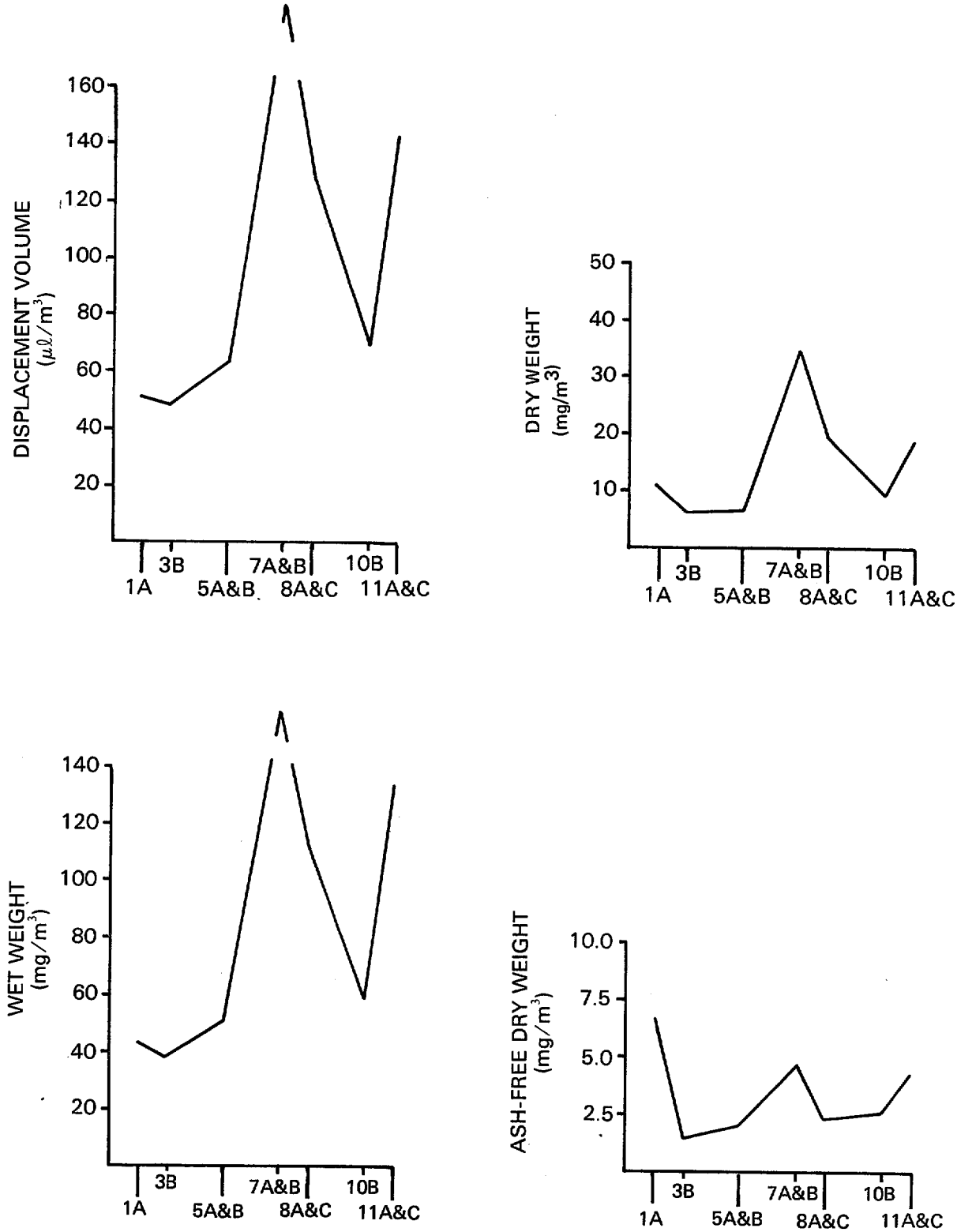

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Figure 2-3. >202μ Net Plankton Average Annual Standing Stock by Station

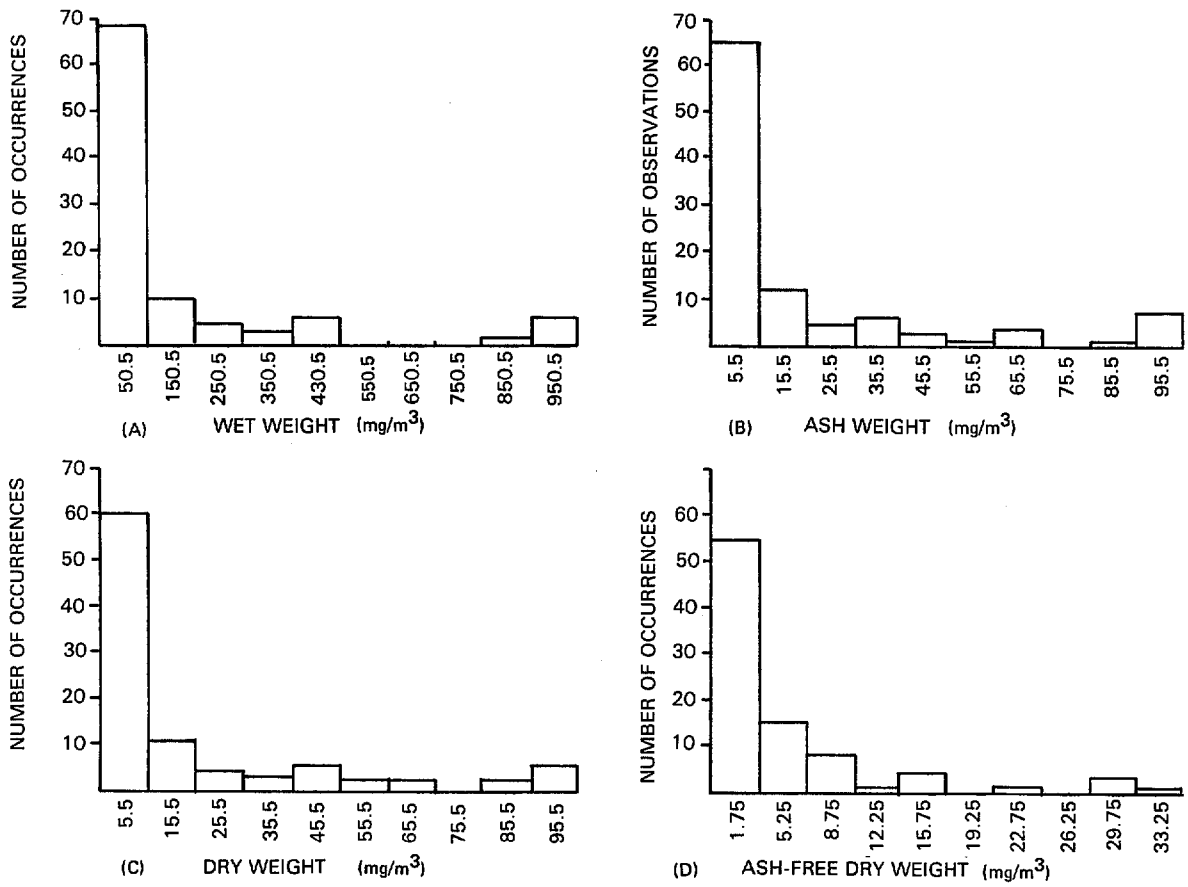

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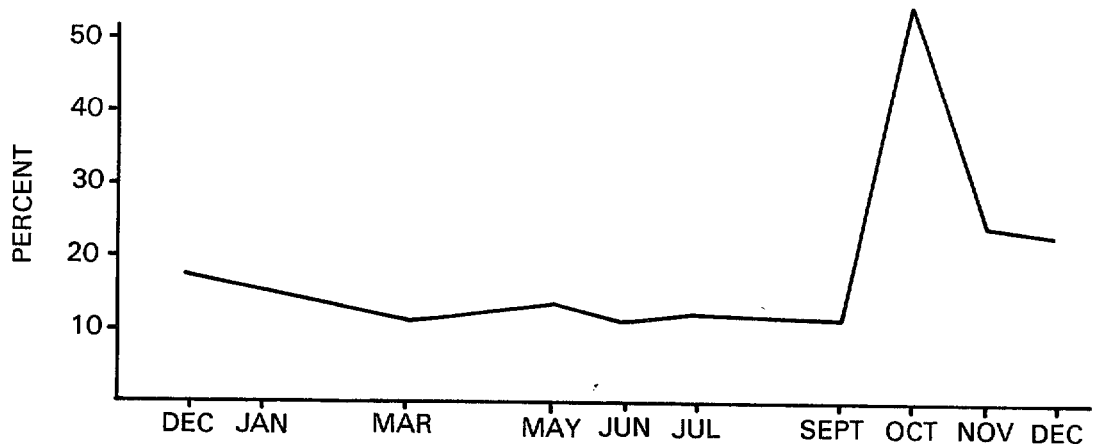
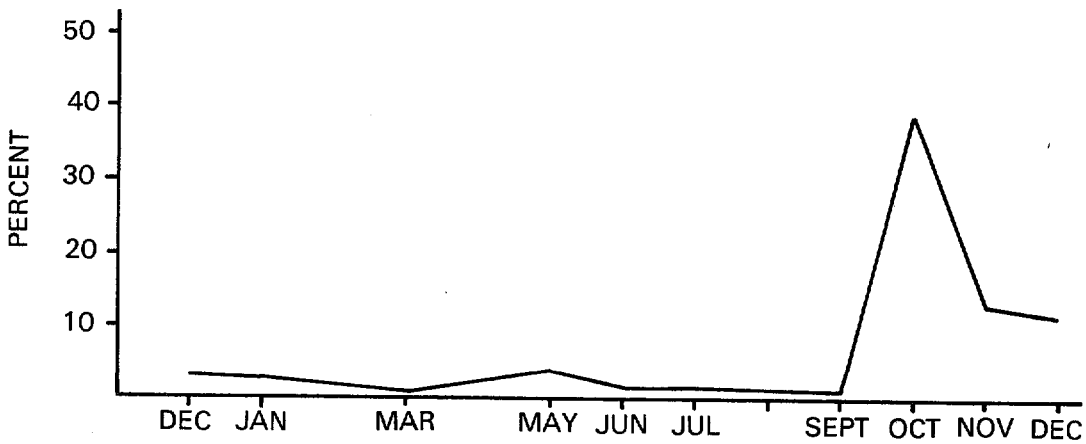
Figure 2-4. Frequency Distributions of Biomass Values for Upper Chesapeake Bay Zooplankton



(A) DRY WEIGHT VS WET WEIGHT



(B) ASH-FREE DRY WEIGHT VS WET WEIGHT

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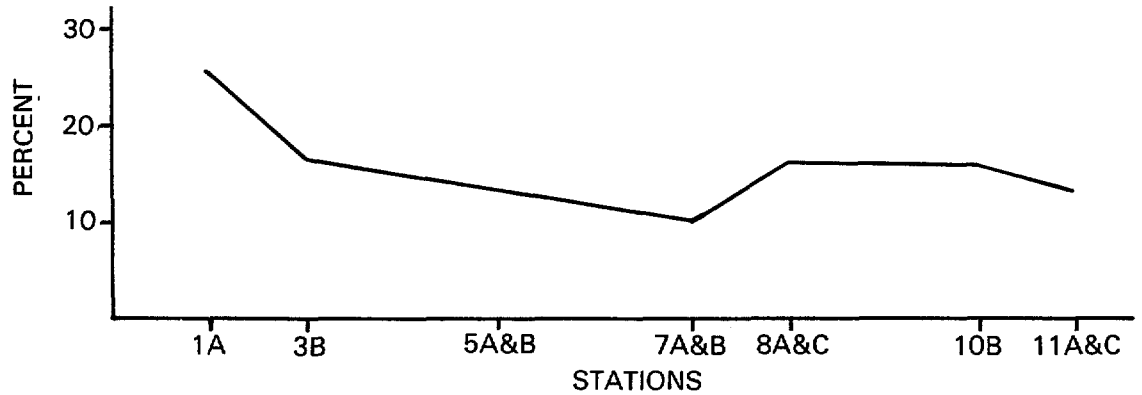
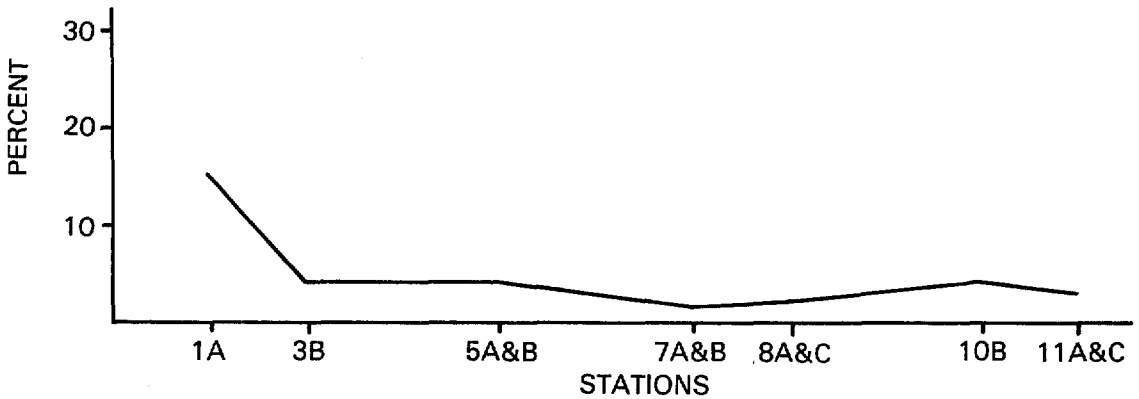
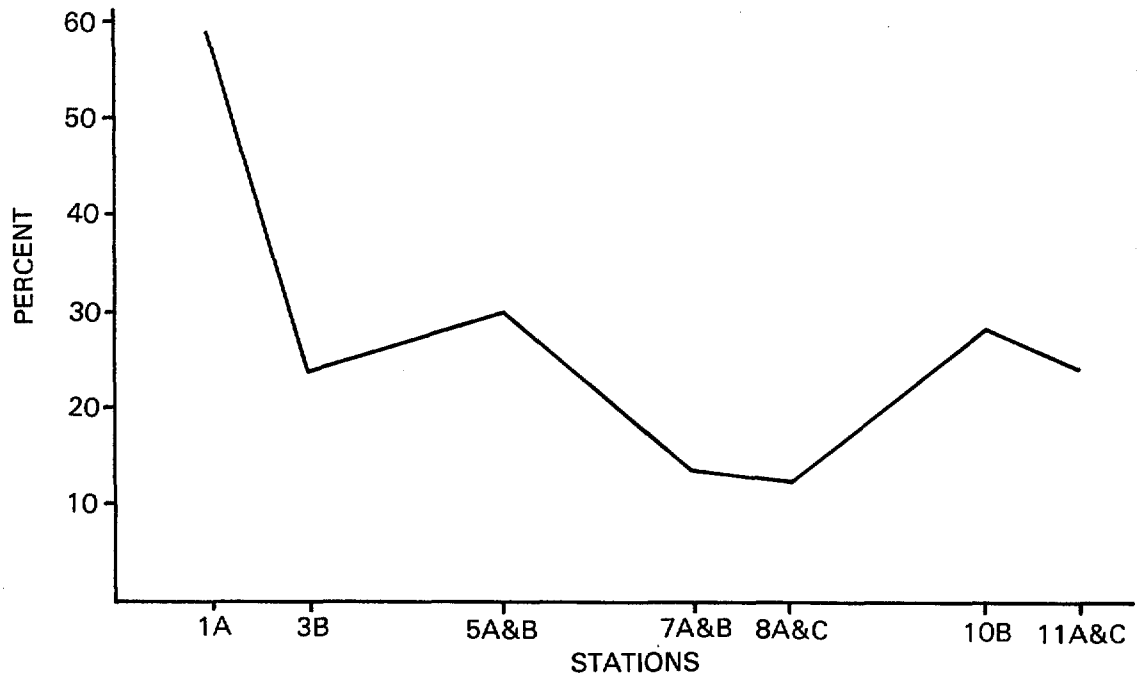
Figure 2-5. Biomass Ratios of Average Monthly Data



(A) DRY WEIGHT VS WET WEIGHT



(B) ASH-FREE DRY WEIGHT VS WET WEIGHT



(C) ASH-FREE DRY WEIGHT VS DRY WEIGHT

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Figure 2-6. Biomass Ratios for Principal Stations Sampled Throughout The Upper Chesapeake Bay

A total of 90 different specific forms were identified and counted from the 51 samples analyzed for taxonomy. By far the most dominant groups were the copepods (27 species) and the cladocerans (20 species). All recognizable forms were categorized and counted during the procedure, resulting in sometimes significant contributions by non-planktonic forms. The second most dominant form isolated in the 202-μ net tows were oak leaf hairs which sometimes accounted for more than 50 percent of the total count from the upper bay samples (Table 2-6). Interestingly, this plant debris is found mostly in the northern bay (Stations 1A and 5A). Numerically, the total number of organisms per cubic meter coincides with the trend of increasing biomass from the head of Chesapeake Bay down to Annapolis and an average of 4,633 organisms per cubic meter recorded in the Baltimore harbor waters.

Species diversity was computed by two methods (Tables 2-7 and 2-8). The highest average diversity occurs in December throughout the upper bay, and the lowest diversity occurs in March when biomasses are at maximum concentrations. On a station basis, the highest species diversity occurs at Station 1A, and the lowest diversity is at the lowermost stations (Stations 10B and 11A). The average numbers of species at each station indicate a similar trend (Table 2-9). While these observations are fairly predictable by making comparisons of salinity profiles throughout the upper bay, numbers of species and individual organisms are almost inversely related to the biomass standing stocks.

Data from stations in the upper bay were compared for organism community relationships (Figure 2-7). These community correlation coefficients show four basic communities of planktonic organisms in the upper Chesapeake Bay. The first, basically a fresh water community, extends from Havre de Grace (Station 1A) to Turkey Point (Station 3B). The second community is in the vicinity of Pooles Island (Station 5A), and the third community seems restricted to the Baltimore harbor area, where salinities average 5°/oo and *Eurytemora affinis* overwhelmingly dominates. The fourth community seems to be located below the Patapsco influence and down to the Severn River area (Station 11A).

Presentation and discussion of the chlorinated hydrocarbon levels found in the zooplankton samples will be found in Chapter 6, Biochemistry.

2.3 Benthic Investigations

Evaluation of the benthos with respect to chlorinated hydrocarbons from upper Chesapeake Bay waters was to meet three basic objectives:

(1) Through selective field sampling, determine the quantitative residual chlorinated hydrocarbon content within indigenous benthic molluscs.

(2) Based on the results of field investigations, experimentally evaluate potential mechanisms of biological uptake.

(3) Working from laboratory tests, conduct *in situ* experimentation atop a productive shellfish bar to verify hypothetical paths of chlorinated hydrocarbon transfer to shellfish.

These objectives were carried out in three phases. The first consisted of field sampling for chlorinated hydrocarbon analysis of upper bay populations of benthic organisms, mainly molluscs. After the analyses of the field samples, laboratory experimentations were designed and undertaken. The final phase of the benthic investigation involved *in situ* tests with commercially-sought shellfish, utilizing as design parameters the results of the field and laboratory work.

TABLE 2-6. DOMINANT FORMS FROM UPPER CHESAPEAKE BAY

TAXA	Percentage of Occurence
Acartia tonsa	98.03
Oak Leaf Hairs	84.31
Barnacle Nauplii	82.35
Podon leucarti	76.47
Eurytemora affinis	74.50
Polychaete Larvae	74.50
Prionocypris longiforma	72.54
Daphnia spp	50.98
Bryozoan Floatoblast	49.01
Cyclops spp (A)	45.09
Bosmina longirostris	45.09
Insects	45.09
Cyclopoid Nauplii	41.17
Harpacticus spp (B)	41.17

TABLE 2-7. >202 μ NET PLANKTON SPECIES DIVERSITY* BY STATION FOR UPPER BAY

	STATIONS					
Month	1A	5A	7A&B	8A&C	10B	11A
12/73	5.6008	3.1038	—	—	1.5135	1.9339
1/74	—	—	—	—	1.9959	2.0807
3/74	3.4769	2.4769	1.3309	1.2024	1.2513	0.7526
5/74	3.8649	3.3574	1.7948	—	2.2694	1.5320
6/74	4.1626	3.2761	3.0980	—	3.0106	1.7644
7/74	4.0255	1.9274	3.8228	—	2.3564	1.3092
9/74	3.2755	2.5390	2.0203	—	0.8889	2.0372
10/74	—	3.7371	—	—	4.1357	2.0487
11/74	—	2.8001	—	—	1.8546	1.6833
12/74	7.2473	4.7905	—	—	2.5492	2.5045

*See Margalef, 1956.

TABLE 2-8. >202μ NET PLANKTON SPECIES DIVERSITY** BY STATION FOR UPPER BAY

	STATIONS					
Month	1A	5A	7A&B	8A&B	10B	11A
12/73	0.8570	0.2219	—	—	0.4837	0.1094
1/74	—	—	—	—	0.2832	0.3672
3/74	0.3928	0.0533	0.0523	0.2082	0.1958	0.2791
5/74	0.4549	0.6944	—	—	0.3773	0.3805
6/74	0.6277	0.2945	0.3980	—	0.2929	0.3314
7/74	0.2612	0.2171	0.5393	—	0.1108	—
9/74	0.4878	0.0662	0.0288	—	0.0814	0.0262
10/74	—	0.0806	—	—	0.3674	0.04383
11/74	—	0.2185	—	—	0.0816	0.02387
12/74	0.7792	0.4394	—	—	0.3919	0.3271

**See Shannon and Weaver, 1949.

TABLE 2-9. AVERAGE NUMBER OF SPECIES PER STATION FROM UPPER CHESAPEAKE BAY

Station	Average Number of Species
1A	29.57 (± 8.69)
5A&B	22.00 (± 5.17)
7A&B	19.83 (± 5.38)
10B	13.90 (± 4.74)
11A	13.30 (± 4.52)

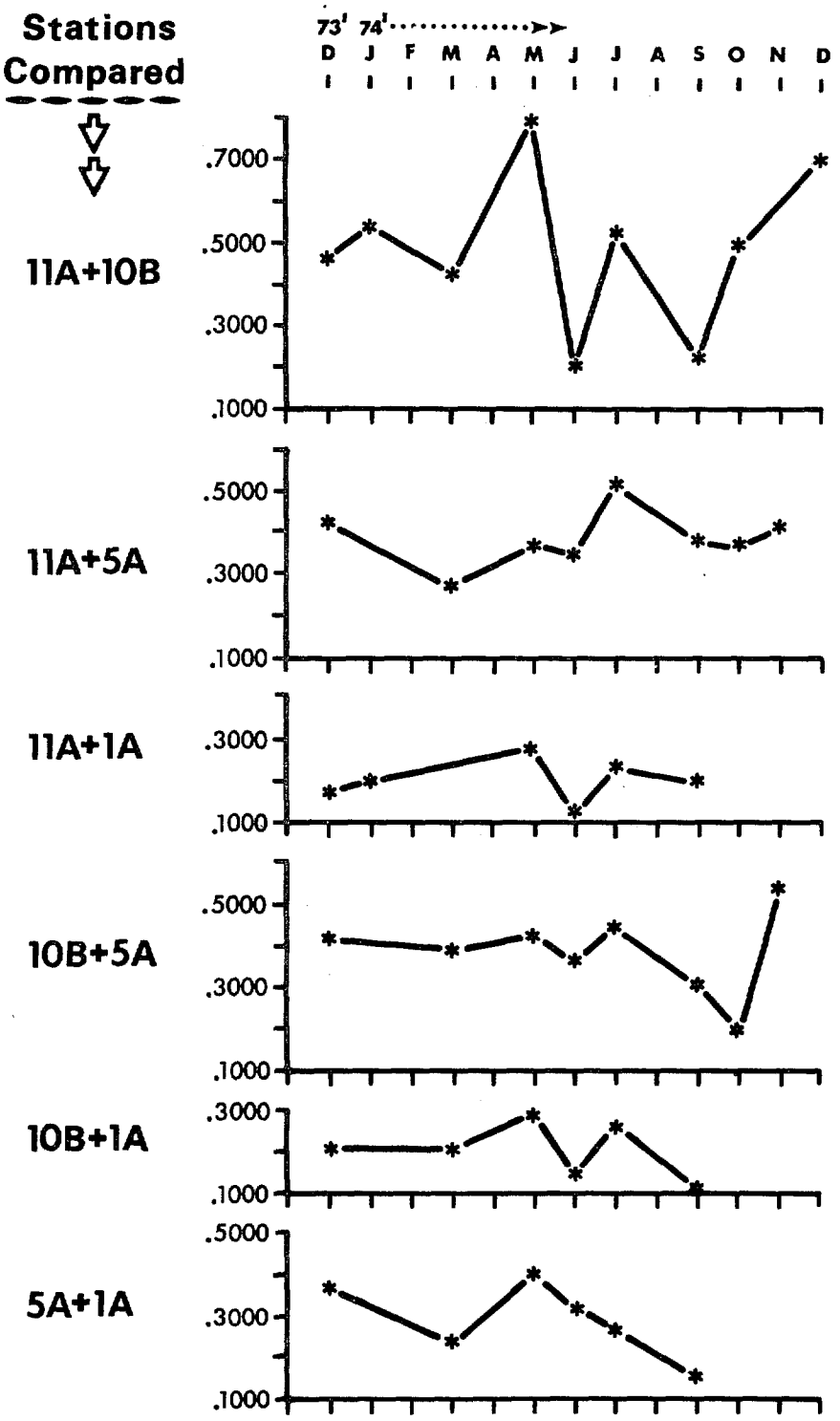

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Figure 2-7. 202μ Net Plankton Investigations Taxonomic Data Analysis: Community Correlations

2.3.1 Field Investigations

Field samples were taken selectively throughout the upper bay during the first phase of the program to collect representative quantities of benthic organisms for evaluation of CHC content. Eight suites of samples were undertaken at stations coincidentally with the plankton and hydrographic measurements (see Figure 2-1) over a period of one calendar year. Approximately 56 individual samples were collected with a modified bottom trawl and a Ponar dredge. The data are recorded in Volume III, Section 4 under the biochemistry section, and the reduced values are given in Table 6-1 in Chapter 6. Section 6.3.4 of that chapter contains further elaboration on the field samples.

2.3.2 Laboratory Experiments

The second phase of the benthic investigations focused on a series of experiments aimed to understand better the natural processes by which chlorinated hydrocarbons become available to commercially important shellfish. The polychlorinated biphenyl (PCB) formulation, Aroclor® 1254, was chosen as the test chemical because of its ubiquity throughout the upper bay field samples. From previous experiments of the Chester River Study (1972), it was believed that substantial amounts of the CHC's present were adsorbed to the fine-grain suspended sediments from which they could be accumulated by shellfish. Work by Huang and Liao (1970) suggested that insoluble, extremely hydrophobic chlorinated hydrocarbons rapidly attach to fine-fraction clay particles. However, their pesticide experiments were conducted with processed (dried) kaolinite, illite, and montmorillonite. While these tests reflected the spectrum of clay composition in suspended sediments, they did not realistically represent the actual conditions under which naturally-existing, fine-fraction suspensoid material is present in an estuarine environment. Therefore, the first series of tests undertaken in the laboratory were to evaluate the capability of PCB 1254 to adsorb on naturally occurring suspended sediments from the upper Chesapeake Bay.

Samples of natural clay sediments taken off Windmill Point in the Chester River were brought to the laboratory wet, where tests were made for percent organic composition and analyses were made for grain-size fraction. Combustion tests on the clays revealed that they contained less than 15 percent organic matter, and the sizes of most particles were found to be in the 0.6 to 1.5 μ range by Coulter counter analysis. To test the ability of this clay sediment to bind PCB 1254, experiments were performed as described below.

The clay experiments consisted of pipetting a few tenths of a milliliter of an acetone solution of PCB 1254 (of sufficient strength to give a resultant concentration of 100 μg/l in the test chamber) into one liter of filtered bay water containing added clay while mixing the water vigorously with a teflon-coated magnetic stirring bar. After immediately withdrawing a 100-ml sample (200-ml sample in the 1,000 mg/1 test), the system was sealed; it was reopened periodically during the ensuing five hours to remove additional subsamples. The 100-ml (or 200-ml) aliquots were immediately filtered through a glass-fiber filter, and both the filters and filtrates were reserved for PCB analysis. Five tests were conducted using suspended sediment concentrations of 50, 100, 500, and 1,000 mg/1 and the control containing no added suspended sediment. The filters containing the suspended sediments and the filtrates were extracted, and the extracts were analyzed for PCB 1254 using gas-liquid chromatography (Chapter 6).

Results of these tests are shown in Table 2-10 and in Figure 2-8. In the first series of tests, it was observed that 44.7 percent of the total 100 μg of PCB was lost when it was injected directly into filtered bay water and mixed for approximately 20 hours (Figure 2-8a).

Repeated tests documented this high loss of PCB. After systematic examination of the probable losses which might be incurring at each step in the procedure, it was concluded that a major part of the loss might be a result of irreversible adsorption of the PCB to the walls of the glass test chamber.

Table 2-10 presents the materials balance for the clay-PCB binding tests in which the amounts of PCB recovered from the clay and the water samples are recorded separately and then as a total. The 200 ml of clay-containing water remaining in each chamber at the end of each test was extracted in the chamber, thereby extracting the walls of the chamber also. The amounts of recovered PCB were added arithmetically to the amounts from the time series samples to derive the percent error, (i.e., the percent of the total PCB remaining unaccounted).

TABLE 2-10. RESULTS OF LOW PERCENTAGE ORGANIC CLAY ADSORPTION EXPERIMENT WITH PCB–1254

Clay Concentration (mg/l)	PCB–1254 RECOVERED			Percent Error*
	Water	Clay	Total	
Control	33.6	2.0	35.6	42.8
50	14.6	18.2	32.8	44.3
100	31.3	16.8	48.1	23.3
500	17.8	30.9	48.7	29.4
1,000	6.5	43.4	49.9	31.7

*Includes Jar Water Recoveries.

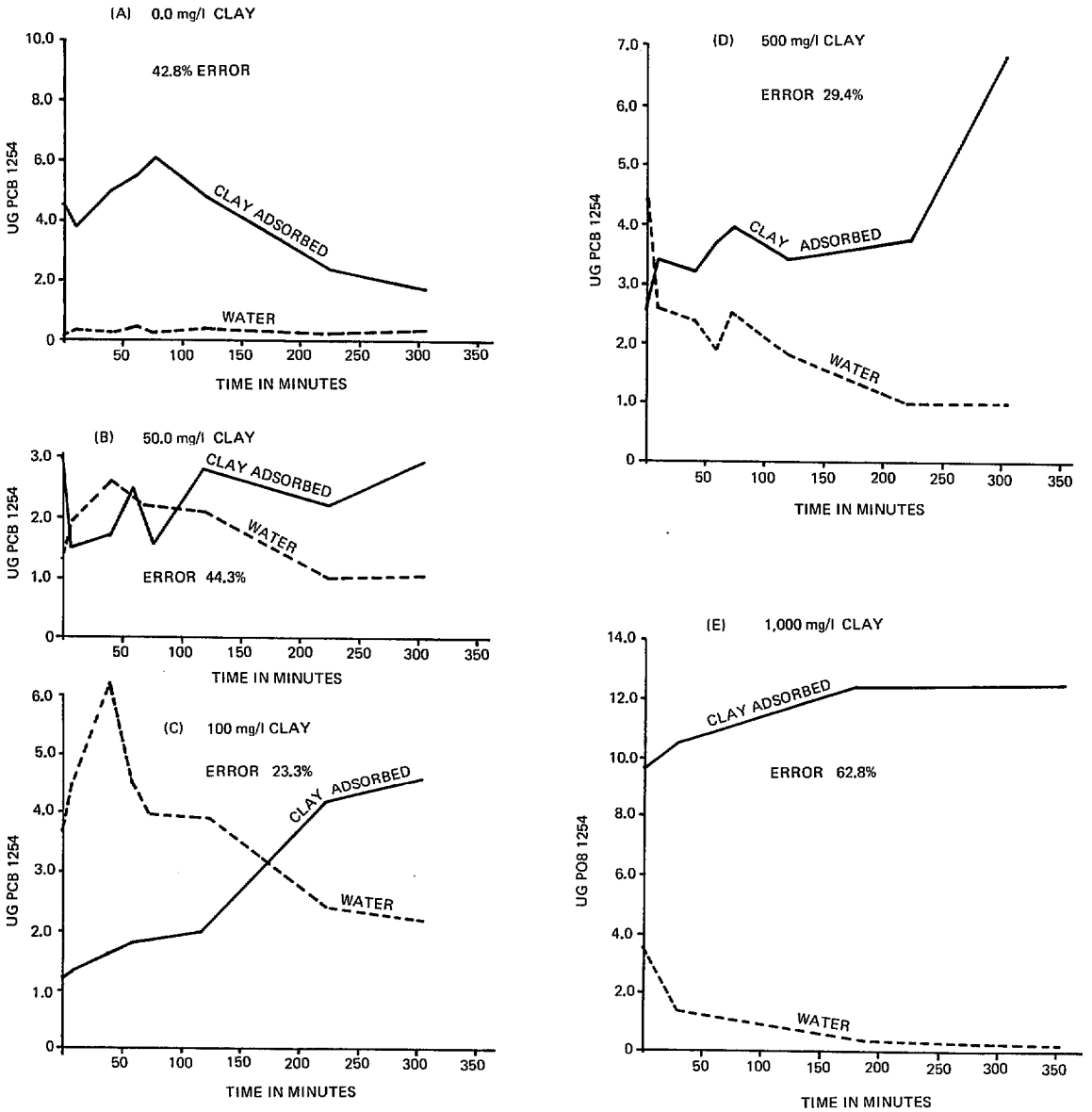

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Figure 2-8. Low Percentage Organic Clay Adsorption With PCB 1254

TABLE 2-10. RESULTS OF LOW PERCENTAGE ORGANIC CLAY ADSORPTION EXPERIMENT WITH PCB–1254

Clay Concentration (mg/l)	PCB–1254 RECOVERED			Percent Error*
	Water	Clay	Total	
Control	33.6	2.0	35.6	42.8
50	14.6	18.2	32.8	44.3
100	31.3	16.8	48.1	23.3
500	17.8	30.9	48.7	29.4
1,000	6.5	43.4	49.9	31.7

*Includes Jar Water Recoveries.

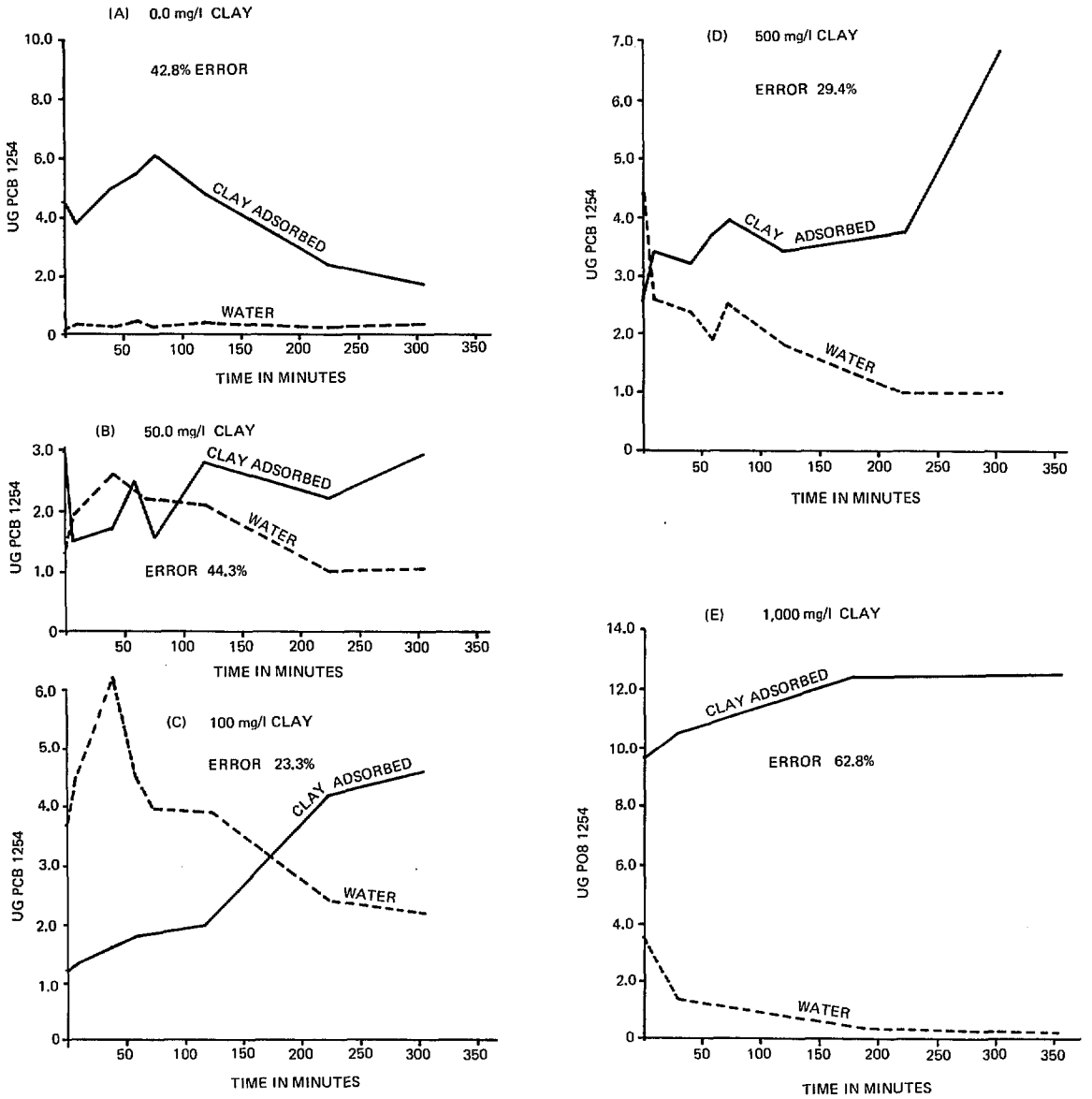

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Figure 2-8. Low Percentage Organic Clay Adsorption With PCB 1254

It is important to note that when no clay was added to the water, very little of the PCB was recovered on the filter (Figure 2-8A). This point is critical to the experiment, because it suggests that the PCB retained on the filters from the tests in which clay was added was really adsorbed to the clay particles rather than the glass-fiber filter.

The plots of the data in Figures 2-8A through 2-8E do not depict smooth curves one might expect from such experiments—possibly indicating random errors in the analytical procedures. A few tentative observations can be made, however. The clay-suspended sediments appear to bind a large part of the added PCB. Apparently, a major part of the adsorption takes place almost immediately, all of the clay samples taken just after addition of the PCB are well on the way to the maximum adsorption observed. Except for the earliest sample in the lowest clay test (which may be an incorrect value), the amount of this initial adsorption increases as the concentration of clay increases. The adsorption process as a whole does not appear instantaneous, however; at this high PCB level, a considerable amount of PCB appears to be free in the water even after five hours of exposure to the clay. As will be seen later from the *in situ* experimental data, the adsorption of PCB by the naturally occurring suspended sediments in bay water appears somewhat different.

In all of the exposures, whether clay was present or not, 23 to 44 percent of the PCB 1254 was lost—more than can be accounted through analytical errors. A part of the PCB seems (1) to become irreversibly attached to the clay, (2) to become irreversibly attached to the glass walls of the test chambers, (3) to volatilize, or (4) perhaps experience a combination of all three.

2.3.3 *In Situ* Experiments:

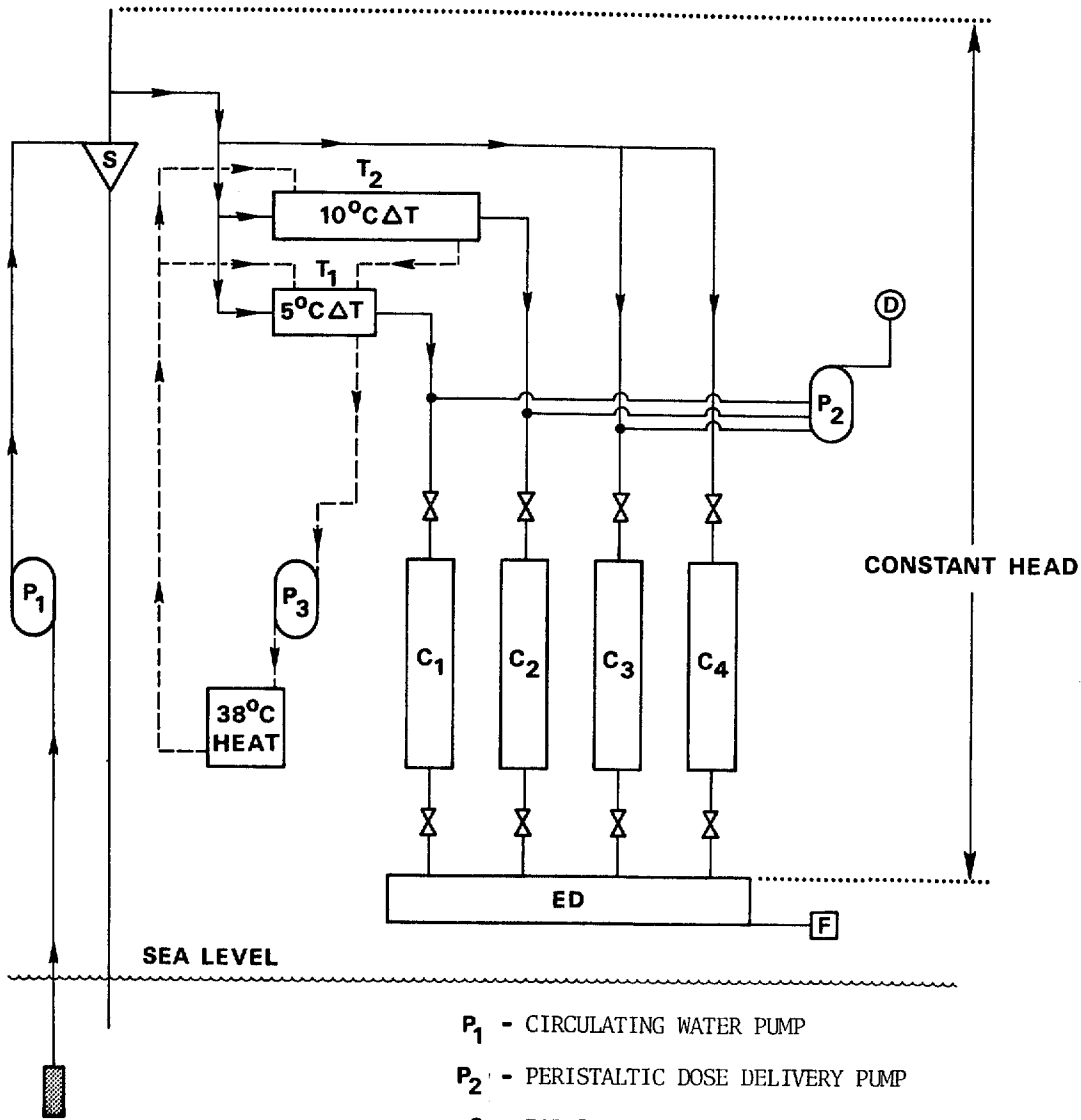
As a climax to the program of field and laboratory biological experiments with chlorinated hydrocarbons, a series of *in situ* tests were designed to assess the reality of background environmental measurements from the field collections and to examine the hypothesized predications of the laboratory experiments. The *in situ* work was designed to conduct realistic dosing experiments using bottom waters taken directly from an active oyster bar and adjusting specific physical parameters to optimize and enhance shellfish pumping characteristics.

The *in situ* experimental apparatus consisted of an open-cycle, once-through seawater circulation system (Figure 2-9) installed in a laboratory module placed on the *R/V SEARCHSTAR* (Figure 2-10A). A high pressure particle separator (Figure 2-10B) in the system was capable of removing particles greater than 20μ from the water at 24 psi. Two modified impervious graphite shell and tube heat exchangers were constructed for raising ambient water temperatures by increments of 4°C and 8°C to increase the pumping rates of the test animals. This variable open-flow, closed-chamber system (Figure 2-10C) was stepped down to produce a flow rate of approximately two liters of water per minute through each test chamber with a residence time of approximately 26 minutes. Using previous calculations by Pratt (1933) and others, it is estimated that the system will circulate more than ten times the volume of water required by the organisms at the maximum operating temperatures. It also provides sufficient time for the chlorinated hydrocarbons to react with ambient suspended sediments and to produce a measurable adsorption to suspended particulates.

Table 2-11 shows the adsorption of PCB 1254 to the suspended sediments during the *in situ* dosing experiments.

Twenty parts per billion (ppb) was selected as a PCB 1254 dose concentration, based on the biochemical results of the field samplings and laboratory experimental tests. Four chambers each containing 12 oysters and 12 clams were prepared for exposure. Selection of organisms for testing was based on comparing data from several series of biomass analyses including displacement volume, displacement wet weights, wet weights, and pseudofeces measurements. Tables 2-12 and 2-13 show the results of this sorting procedure and Figure 2-11 shows the linear regressions for the oysters and clams selected for experimentation.

The clams were water-injected into a sand substrate in 250-ml breakers to reduce the hydrostatic stresses on these infaunal organisms. The test animals were then placed in their respective tubes for *in situ* experimentation. After a 48-hour period for acclimation of the test animals to the tube chambers and elevated temperatures, the laboratory module was towed to Station 11A off Hacketts' bar.



P_1 - CIRCULATING WATER PUMP

P_2 - PERISTALTIC DOSE DELIVERY PUMP

S - PARTICLE SEPARATOR (<20μ @ 24 PSI)

T_1 - 5°C ΔT HEAT EXCHANGER

T_2 - 10°C ΔT HEAT EXCHANGER

100°F HEAT SOURCE

D - CHEMICAL DELIVERY RESERVOIR

$C_{1...4}$ - ANIMAL TEST CHAMBERS

ED - EFFLUENT DISCHARGE POINT

F - FILTRATION CLEANUP

P_3 - HOT WATER CIRCULATION PUMP

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Figure 2-9. In Situ Experimental Laboratory System

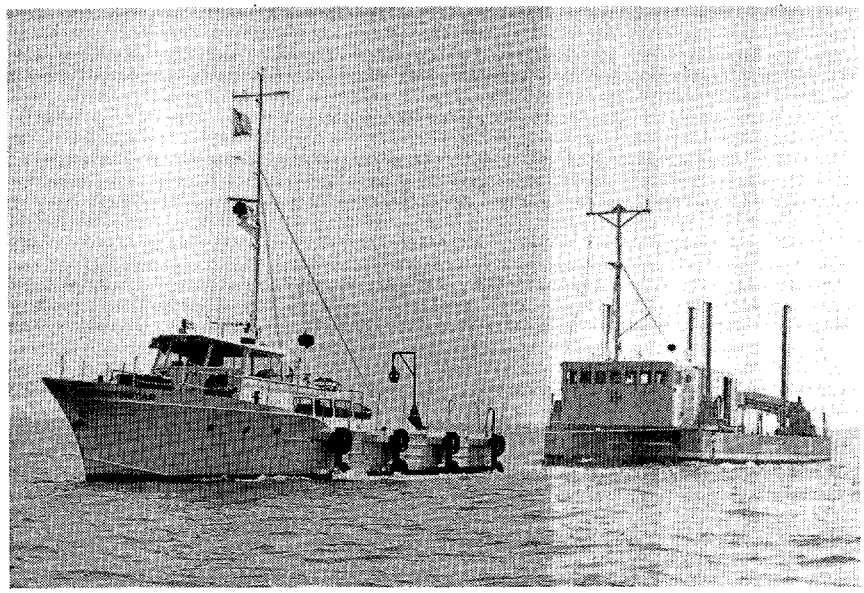

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Figure 2-10A. Laboratory Module on R/V SEARCHSTAR being Towed to Station by R/V NORTHSTAR

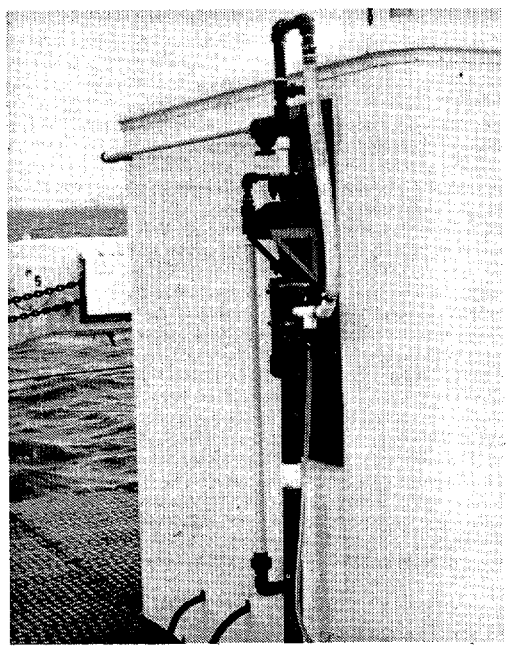

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Figure 2-10B. High Pressure Particle Seperator Used in the In Situ Dosing Experiments

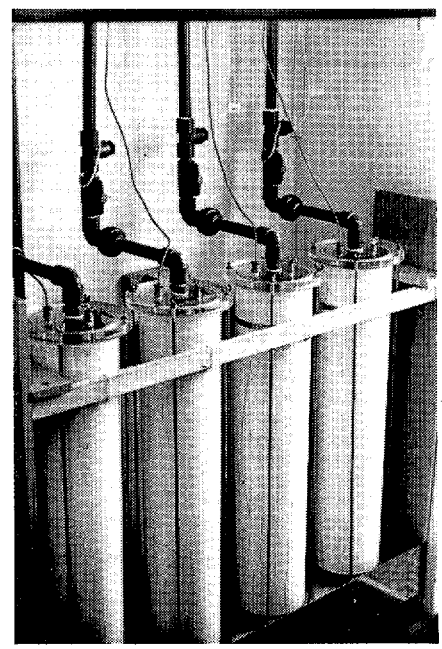

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Figure 2-10C. Organism Test Chambers Having a Two-Liter per Minute Flow Rate as Used in the In Situ Dosing Experiments

TABLE 2-11. PCB-1254 ADSORBED TO SUSPENDED SEDIMENTS DURING IN SITU EXPERIMENT

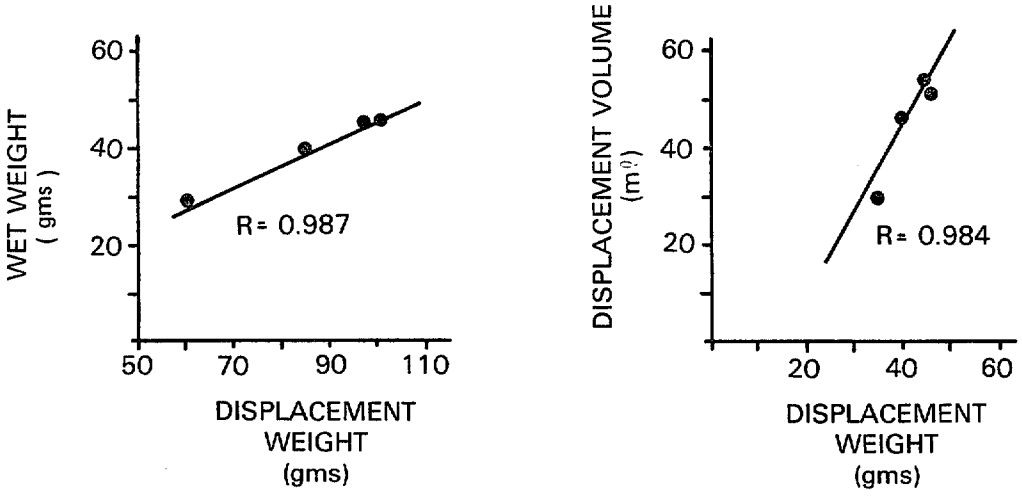
Tank No.	Time (Hours)	ug/l PCB–1254 Filter	Water
2	24	3.6614	0.4649
2	48	5.2834	0.0877
2	96	1.7805	0.1986
2	144	5.0721	0.0001
3	24	2.9164	0.9351
3	48	2.9270	0.1558
3	96	1.0461	0.0760
3	144	2.2560	0.0389
4	24	3.1383	0.2377
4	48	4.9558	0.8030
4	96	0.8559	0.0861
4	144	8.0308	0.2958

TABLE 2-12. OYSTER BIOMASS SORTING EVAULATIONS FOR IN SITU EXPERIMENTATIONS

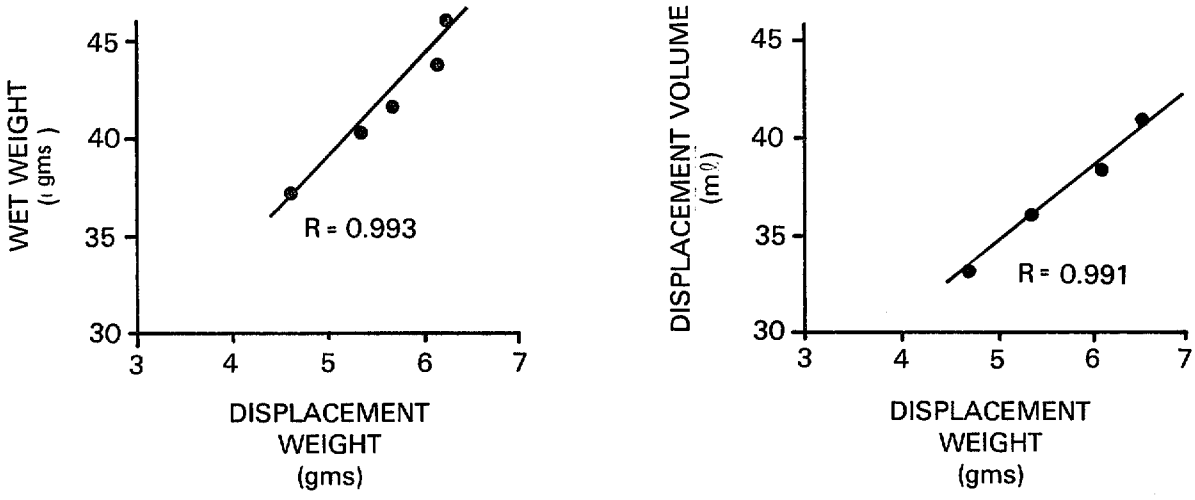
	TUBE NUMBER 1	2	3	4
Displacement Volume	54.53 (±12.32)	51.29 (± 9.58)	45.67 (± 9.03)	35.18 (±11.90)
Displacement Weight	45.87 (± 9.18)	45.92 (±10.46)	40.58 (±11.24)	29.91 (±11.09)
Wet Weight	101.75 (±16.51)	95.93 (±18.87)	85.54 (±19.52)	64.23 (±22.31)
Composite Meat Weight	29.75 (± 7.73)	27.83 (± 7.05)	21.67 (± 3.14)	14.80 (± 3.72)

TABLE 2-13. CLAM BIOMASS SORTING EVALUATIONS FOR IN SITU EXPERIMENTATIONS

	TUBE NUMBER			
	1	2	3	4
Displacement Volume	54.53 (± 12.32)	51.29 (± 9.58)	45.67 (± 9.03)	35.18 (±11.90)
Displacement Weight	45.87 (± 9.18)	45.92 (±10.46)	40.50 (±11.24)	29.91 (±11.09)
Wet Weight	101.75 (± 16.51)	95.93 (±18.87)	85.54 (±19.52)	64;23 (±22.31)
Composite Meat Weight	29.75 (± 7.73)	27.83 (± 7.05)	21.67 (± 3.14)	14.80 (± 3.72)



(a) OYSTERS



(b) CLAMS

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Figure 2-11. Regressions on Biomass Animal Sorting Evaluations

The PCB 1254 dosing was initiated then at a calculated concentration of 20 ppb with a bay water flow rate of two liters per minute. One milliliter per minute of 40 ppm solution of PCB 1254 in 20-percent acetone in water was pumped into the inlet water lines of exposure Tubes 2, 3 and 4. (The control tube, Tube 1, received 1.0 ml per minute of 20-percent acetone in water.) A fourth channel of the pump delivered 1.0 ml per minute of the PCB solution to a holding bottle for later analysis. Temperatures, flow rates, and PCB dose rate were monitored every two hours, and the reduced results were recorded in Table 2-14.

From examining these data, one observes the daily average temperature varied only 0.65°C, and the flow rates were within ±64 ml/min during the entire 144 hours of dosing. Measured concentrations of PCB in the holding bottle were within 10 percent of the calculated dose rate. Sequential measurements were made by analyzing organisms, suspended sediments, and filtrate waters (Table 2-15). The time sequences arbitrarily chosen for measurement were: (1) just before dosing began, (2) at 24, 48, and 96 hours after initiation of dosing, and (3) a final measurement 48 hours after cessation of dosing at 144 hours.

In view of the high levels of PCB used in this experiment, the analyses were not carried to such extremely low levels as with the environmental samples. The values reported for PCB in the filtrate water and filtered suspensoids below the 0.2 μg/1 level probably are not significant and do not necessarily represent positive confirmations of the presence of PCB 1254. The PCB was equal to or less than those values, if it was present at all. For this reason, one should not conclude that the bay water passing through the control tube contained more PCB free in the water than on the suspended sediments.

Referring to Table 2-15, one can notice several interesting things about the administered PCB. In most cases, the values for PCB free in the water (i.e., passing through the filter) fall below the 0.2 μg/l significance level. The lack of any trend or apparent relationship between the values which rise above this level cause one to suspect random analytical errors for these positive values. At any rate, one seems justified in concluding that very little and perhaps none of the PCB 1254 present was free in the water, but it was adsorbed on the suspended sediments in the water instead.

These data also indicate that the adsorption of PCB 1254 by the suspended sediments was more complete and more rapid than by the clay sediments employed in the laboratory tests. Perhaps having the PCB concentration closer to its water solubility (about 10 ppb) accelerated the adsorption although less suspended sediment was available. Whatever the case, most of the 20 ppb PCB 1254 added was not found in the water and suspended sediments leaving the exposure tubes. Although analytical errors may be responsible for some of the irregularity, why the recovered amounts of PCB varied so widely and usually represented less than 25 percent of the amounts added remains a mystery.

It seems, however, that the PCB available during dosing was adsorbed to fine-fraction particulate matter and that the resulting uptake by organisms must come from ingestion of fine particulates, although they may not be viable organic matter. The oyster data suggest that, although these organisms can scrub PCB from suspended sediments effectively, they accumulate very little PCB when water temperatures are below 10°C (Figure 2-12). However, when water temperatures exceed 10°C, oysters can accumulate five to ten times more PCB. Further, these animals at temperatures above 10°C were able to purge 44 percent of the PCB within 48 hours after cessation of exposure, but the oysters exposed to temperatures below 10°C did not show any significant changes in PCB level.

The results from exposing clams did not show the same trends as the results from exposing oysters; although, conditions were exactly the same (Figure 2-13). The clams seemed to accumulate PCB even when ambient temperatures were around 4.7°C. However, at this temperature their body burden stabilized at about 2 ppm within 48 hours of dosing and dropped to only 1 ppm 48 hours after dosing was terminated. The organisms exposed at elevated temperatures generally accumulated higher concentrations of PCB, but no definite pattern of uptake was observed. From these results, it is difficult to identify any trend with regard to PCB uptake by clams. However, there appears to be a significant accumulation of PCB within the first 48 hours of exposure, followed by fluctuations on the order of 7 ppm in PCB levels in the clams.

TABLE 2-14. PHYSICAL PARAMETER MEASUREMENTS DURING IN SITU DOSE EXPERIMENT CONDUCTED MARCH 3 THRU 17, 1974 AT STATION 11A

		DATE						
		3/11	3/12	3/13	3/14	3/15	3/16	3/17
Temperature (°C)	No. 1	4.71 (±0.31)	4.60 (±0.11)	4.92 (±0.25)	4.70 (±0.12)	4.74 (±0.28)	4.89 (±0.47)	5.12 (±0.19)
	No. 2	4.77 (±0.32)	4.65 (±0.10)	4.95 (±0.24)	4.73 (±0.11)	4.76 (±0.28)	4.92 (±0.47)	5.16 (±0.17)
	No. 3	12.57 (±0.57)	12.26 (±0.40)	12.47 (±0.23)	12.51 (±0.42)	12.50 (±0.21)	12.63 (±0.54)	12.86 (±0.16)
	No. 4	8.74 (±0.25)	8.60 (±0.07)	8.91 (±0.23)	8.72 (±0.18)	8.73 (±0.15)	8.91 (±0.48)	9.13 (±0.20)
	Air	4.64 (±0.69)	4.79 (±0.72)	6.96 (±1.78)	2.56 (±1.42)	4.07 (±1.74)	5.24 (±2.62)	5.07 (±1.92)
	Surface	4.47 (±0.40)	4.29 (±0.14)	4.56 (±0.22)	4.49 (±0.11)	4.50 (±0.27)	4.56 (±0.39)	4.84 (±0.15)
Flow Rate (l./min.)	No. 1	1.990 (±0.062)	2.040 (±0.061)	2.003 (±0.049)	1.953 (±0.011)	1.949 (±0.020)	1.963 (±0.010)	1.924 (±0.025)
	No. 2	1.997 (±0.067)	2.046 (±0.014)	2.021 (±0.020)	1.942 (±0.008)	1.942 (±0.059)	2.097 (±0.018)	2.058 (±0.024)
	No. 3	1.962 (±0.017)	2.059 (±0.064)	2.088 (±0.024)	1.953 (±0.029)	1.979 (±0.033)	2.003 (±0.021)	1.995 (±0.008)
	No. 4	1.976 (±0.012)	1.970 (±0.012)	1.955 (±0.013)	1.932 (±0.009)	1.943 (±0.009)	1.971 (±0.017)	1.986 (±0.008)

TABLE 2-15. DATA FROM IN SITU EXPERIMENT CONDUCTED MARCH 3 THRU 17, 1974 AT STATION 11A

	Tube No.	Time (Hours)	Suspended Sediments (mg/l)	PCB in Water (μg/l)	PCB on Filters (μg/l)	PCB in Clams (ppm)	PCB in Oysters (ppm)
Control	1	0	19.47	0.174	0.000	0.072	0.084
	1	24	10.53	0.171	0.004	0.006	0.009
	1	48	8.27	0.030	0.010	0.021	0.012
	1	96	11.40	0.006	0.119	0.054	0.042
	1	144	12.43	0.005	0.072	0.103	0.030
	1	192	10.93	0.000	0.005	0.048	0.028
Ambient Temperature	2	0	18.04	—	0.010	0.045	0.085
	2	24	12.77	0.465	3.661	0.419	0.049
	2	48	8.43	0.088	5.283	1.920	0.026
	2	96	9.30	0.199	1.781	1.850	0.089
	2	144	13.63	0.001	5.072	1.910	0.186
	2	192	11.83	0.001	0.003	1.100	0.050
+8°C	3	0	19.60	0.137	0.009	0.199	0.100
	3	24	8.60	0.935	2.916	1.770	0.128
	3	48	11.83	0.156	2.927	5.520	0.462
	3	96	11.33	0.075	1.046	11.500	4.800
	3	144	13.27	0.039	2.256	4.810	22.700
	3	192	13.70	0.000	0.001	5.300	9.000
+4°C	4	0	18.04	0.063	0.004	0.169	0.063
	4	24	7.50	0.238	3.138	2.330	0.414
	4	48	7.70	0.803	4.956	7.780	0.389
	4	96	7.93	0.086	0.856	3.040	1.720
	4	144	11.17	0.296	8.031	9.970	0.889
	4	192	10.80	0.001	0.001	4.100	2.400

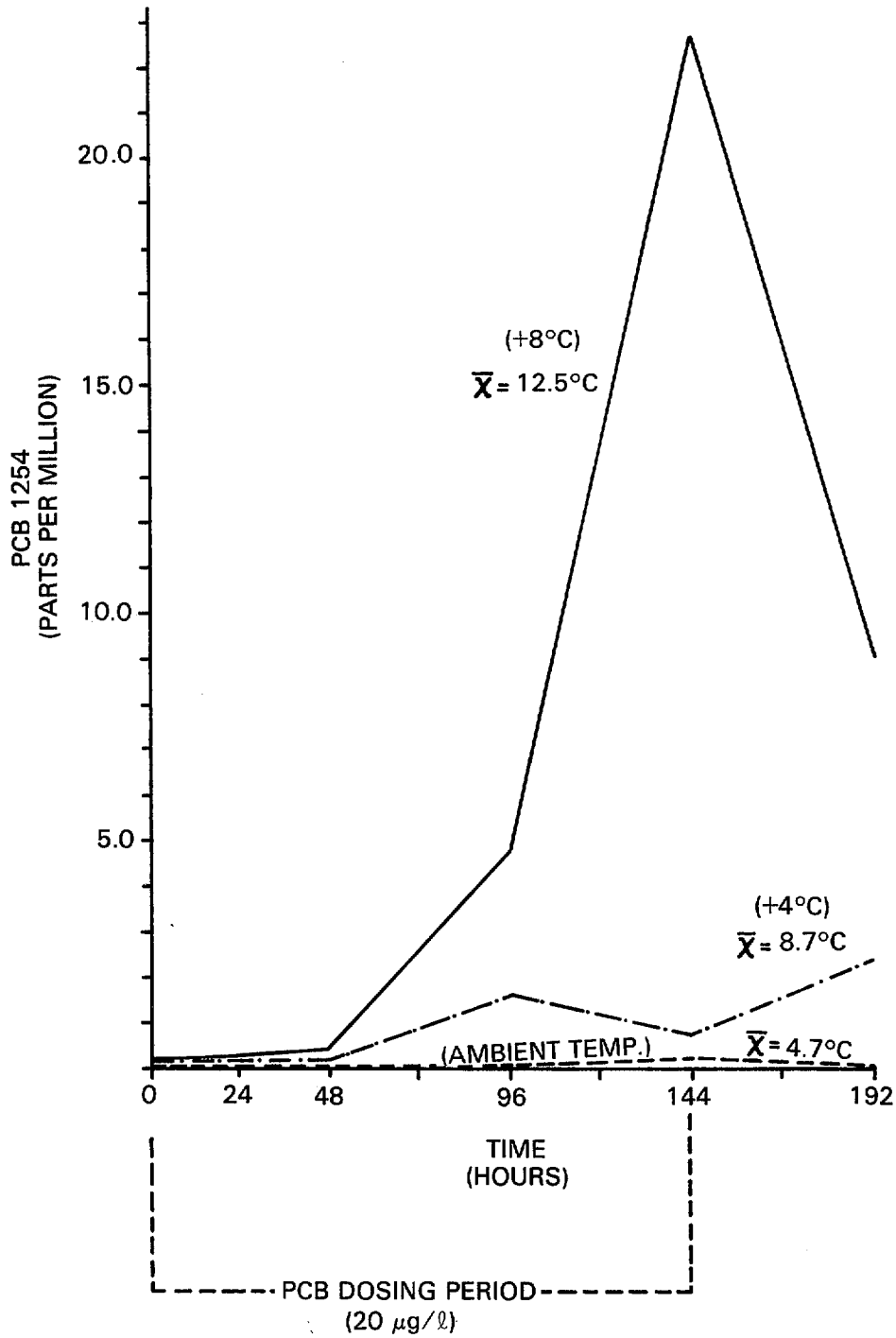

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Figure 2-12. PCB 1254 Uptake by Oysters During In Situ Test

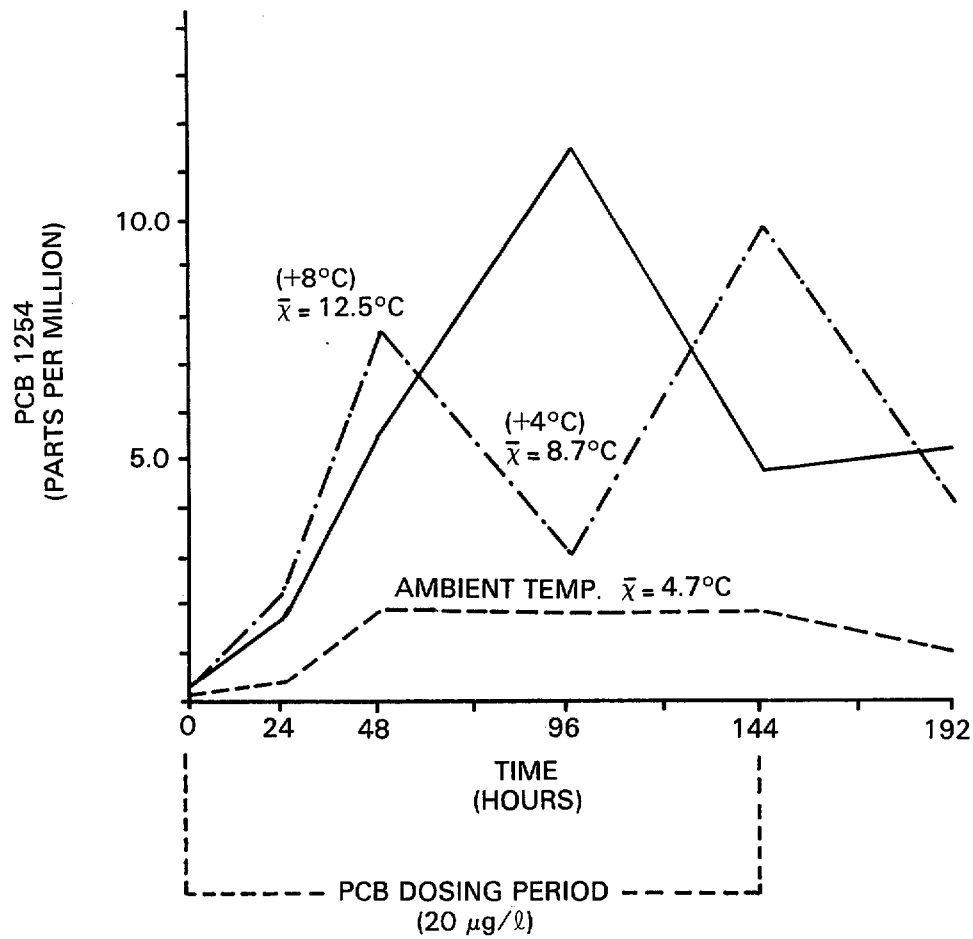

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Figure 2-13. PCB Uptake by Clams During In Situ Test

Several conclusions can be drawn from the oyster and clam *in situ* exposures to PCB. First, it appears that filter-feeding oysters can scrub PCB from suspended particulates, and the amount of uptake is directly related to their pumping rate. Secondly, oysters can purge themselves of PCB within 48 hours if their pumping rate is high. Thirdly, clams, will accumulate PCB through siphon pumping even under winter conditions when temperatures are below 5°C as was demonstrated with chlordane in the Chester River Study. Fourthly, after the clams initially (within 48 hours) accumulate PCB to a concentration of approximately 5 to 6 ppm, there is no identifiable body burden, and levels tend to fluctuate. This does seem consistent with their feeding behavior, and similar observations involving other chemicals have been made.

2.4 Conclusions

The results of the work performed led to certain conclusions which merit the attention of resource managers. While the data supporting these results may not be absolutely and statistically significant, it is the sincere judgment of the principal investigator that the following conclusions can be drawn:

(1) Although, the zooplankton populations of upper Chesapeake Bay are spatially distributed and influenced primarily by seasonal temperature and salinity regimes, zooplankton populations within the Baltimore harbor area are distinct. Based on more than 95 measurements of biomass, stations sampled from the Baltimore harbor area yielded values four to five times greater than those from any other stations in the upper bay.

(2) Zooplankton organism diversity decreases from the head at Havre de Grace down the bay toward Annapolis; yet biomass abundance increases. This means there are more different types of plankton organisms in the upper reaches, but standing stocks there are substantially lower than at the more southerly stations of the upper bay. An hypothesis to explain this phenomenon is that enormous pulses of suspended particulate loads are introduced into the bay from the Susquehanna River and the resulting turbidities depress autotrophic production.

(3) Compared to similar mid-temperate zone estuaries, the upper Chesapeake Bay supports a healthy diversity of species in an abundant zooplankton community. However, contrary to some published data, the dominant organism throughout the upper bay (*Acartia tonsa*) does not have a winter replacement (*Acartia clausi*). This copepod, *Acartia tonsa*, functions as the single most important holoplanktonic species supporting the secondary level of the aquatic food web.

(4) From the data collected during the laboratory experiments, it seems that laboratory results cannot be used to make direct predictions of field situations in estuarine waters with respect to chlorinated hydrocarbon behavior. The behavior of chlorinated hydrocarbons in adsorption to natural sediments in the laboratory is not the same as *in situ*.

(5) The biological effects of chlorinated hydrocarbons on oysters are directly related to their pumping rate and efficiency, which in turn are regulated by the basic environmental parameters of temperature and salinity. For oysters, there appears to be a 10°C threshold below which chlorinated hydrocarbon uptake will not occur and above which concentrations in whole-body tissue can triple within 96 hours of exposure.

(6) The behavior of clams subjected to chlorinated hydrocarbon stresses indicates that there is no definite temperature threshold controlling uptake.

(7) Comparison of oyster and clam *in situ* exposure data indicates that each organism responds differently to chlorinated hydrocarbon stresses and that measurements of source water characteristics may not be sufficient to identify or extrapolate environmental stresses to shellfish by chlorinated hydrocarbons.

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CHAPTER 3

MICROBIOLOGY

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ABSTRACT

The microbiology of suspended particulates, bottom sediments, and water of the upper Chesapeake Bay was examined. Co-transportation of chlorinated hydrocarbons, bacterial indicator organisms, and potential pathogens via suspended sediment was found to occur. The highest total viable counts (TVC) of bacteria in bottom water samples were observed during the winter and spring months; the lowest were observed in July, September, and October. Total viable counts of bottom sediment samples followed essentially the same distribution. The highest recorded counts over the year, obtained at Station 1A, were ten to a hundred times greater than at Stations 5B and 10B.

Most probable number (MPN) levels of total coliforms, fecal coliforms, and fecal streptococci fluctuated seasonally and spatially from Stations 1A to 11A. In general, MPN values decreased from winter to summer, with a gradual increase in the number of indicator organisms in the fall, except that higher MPN values occurred at Station 11A in June through September. Total coliform levels were higher than fecal coliforms and fecal streptococci levels, with the highest MPN values being found in water samples from Station 1A. The entry of total coliforms to the upper Chesapeake Bay via the Susquehanna River appears to be significant. MPN values at all of the sampling stations were lower in bottom sediments than in the water, with relatively less of a seasonal fluctuation occurring in the sediments. Fecal streptococci levels were generally higher than total and fecal coliform levels in bottom sediment.

Approximately 80 percent of the fecal coliforms were *Escherichi coli*, Type I. Station 10B more commonly gave false positive coliform MPN's. More than 80 percent of the fecal streptococci were enterococci.

More than 33 percent of the FC:FS ratios were greater than 4.0 for Station 1A samples, including suspended sediment samples.

The relative proportions of fecal coliforms and fecal streptococci in the total viable counts decreased southwards from Station 1A to 11A during the winter months, were uniform during the spring months, and rose starting in June at Station 11A.

A highly significant proportion of both total viable bacteria and selected indicators of fecal pollution were found associated with particulate matter in the water column. Up to 53 percent of the total viable bacteria in Station 11A water samples was found to be associated with particulate matter.

By means of a non-selective enrichment, *Salmonella enteritidis* was isolated from samples collected during this study. *Clostridium botulinum* Types B and E, *Vibrio parahaemolyticus,* and suspected *Yersinia* sp. also were isolated.

Approximately one to ten percent of the total viable bacterial population was found to be resistant to, and potentially capable of metabolizing PCB 1254. These bacteria were present at all stations.

Greater amounts of humic acid were found in samples collected at Station 1A, and the humic acid concentration decreased from Station 1A to Station 11A.

The oyster, *Crassostrea virginica,* was found to accumulate *Salmonella typhimurium* and *E. coli* at similar rates under controlled, PCB-stressed conditions. However, depuration of indicator organisms, viz. *E. coli,* was reduced under PCB stress, resulting in an artificially improved bacteriological quality of the oyster.

TABLE OF CONTENTS

Paragraph		Page
3.	MICROBIOLOGY	3-1
3.1	Introduction	3-1
3.2	Media and Methods	3-2
3.2.1	Total Viable Counts	3-2
3.2.2	Most Probable Number of Selected Indicator Organisms	3-2
3.2.3	Isolation of Pathogenic Bacteria	3-4
3.2.4	Suspended Sediment Analyses	3-9
3.2.5	Humic Acid Determination	3-12
3.2.6	Nutrient Analyses	3-12
3.2.7	PCB-Metabolizing Bacteria	3-12
3.2.8	Bacteriological Analyses of Oysters	3-16
3.3	Results and Discussion	3-16
3.3.1	Total Viable Counts	3-16
3.3.2	Most Probable Number Index of Indicator Organisms	3-17
3.3.3	Effeciency of Identification of Indicator Organisms	3-22
3.3.4	FC/FS Ratios	3-22
3.3.5	Occurrence of Indicator Organisms Relative to Total Viable Counts	3-22
3.3.6	Association of Bacteria with Particulates	3-24
3.3.7	Isolation of Potentially Pathogenic Bacteria	3-28
3.3.8	Bacteriology of Oysters	3-29
3.3.9	PCB-Metabolizing Bacteria	3-30
3.3.10	Effect of PCB-1254 on Bacterial Activity	3-30
3.4	Conclusions	3-39
3.5	Acknowledgements	3-39
3.6	References	3-40
	APPENDIX 3A	
3A1.	Introduction	3A-1
3A2.	Materials and Methods	3A-2
3A2.1	Culture Conditions	3A-2
3A2.2	Oyster Maintenance	3A-3
3A3.	Results and Discussion	3A-4
3A3.1	PCB Stress and the Accumulation of *E. coli*	3A-4
3A3.2	PCB Stress and the Accumulation of *Salmonella Typhimurium*	3A-8
3A4.	References	3A-13
	APPENDIX 3B	
3B1.	Introduction	3B-1
3B2.	Materials and Methods	3B-2
3B2.1	Sampling	3B-2
3B2.2	Bacterial Enumeration	3B-2
3B2.3	Characterization of Isolates	3B-5
3B3.	Results and Discussion	3B-6
3B3.1	Recovery of *Salmonella*	3B-6
3B3.2	Evaluation of Enrichment Techniques	3B-10
3B3.3	*Salmonella* Characterization	3B-10
3B4.	Acknowledgements	3B-14
3B5.	References	3B-15

LIST OF FIGURES

Figure		Page
3-1	Bacterial Association with Particulates (Light Microscopy 1,000 X)	3-13
3-2	Bacterial Association with Particulates (Light Microscopy 1,000 X)	3-13
3-3	Bacterial Association with Particulates (Light Microscopy 1,000 X)	3-14
3-4	Bacterial Association with Particulates (Light Microscopy 1,000 X)	3-14
3-5	Bacterial Association with Particulates (Light Microscopy 1,000 X)	3-14
3-6	Comparison of the Temporal Distribution of Total Viable Bacterial in Water and Sediment Among Upper Bay Samples	3-18
3-7	Comparison of the Temporal Distribution of Fecal Indicator Organisms in the Bottom Water Among Upper Bay Samples	3-19
3-8	Comparison of the Temporal Distribution of Fecal Indicator Organisms in the Sediment Among Upper Bay Stations	3-20
3-9	Comparison of Waterborne Indicator Organisms in Relation to the Temporal Distribution of Total Viable Bacteria Among Upper Bay Stations	3-21
3-10	Comparison of Sediment-Bound Indicator Organisms in Relation to the Temporal Distribution of Total Viable Bacteria in Upper Bay Sediments	3-23
3-11	Comparative Recovery of Bacteria Associated with the Suspended Sediment at Stations 1A and 11A	3-27
3-12	Effect of PCB 1254 on the Dissimilation of [$U^{-14}C$] Glucose by a Heterogenous Marine Population	3-34
3-13	Oxidation of PCB 1254 by an Estuarine *Pseudomonas* spp	3-36
3-14	Effect of PCB 1254 on Glucose Oxidation by an Estuarine *Pseudomonas* spp	3-37
3A-1	Survival of Total Viable Bacteria (TVC) and *E. coli* in Aquarium Water Under PCB-Stress and Unstressed Conditions. (▲ *E. coli* — PCB Stress, △ *E. coli* — No Stress, ● TVC — PCB Stress, o TVC — No Stress; Oysters Were Removed from the Aquaria at Day 12.)	3A-6
3A-2	Accumulation and Excretion of *E. coli* by Oysters Following PCB Stressed and Unstressed Conditions. (▲ *E. coli* Tissue Accumulation with PCB-Stress; △ *E. coli* Tissue Accumulation with No Stress; ● *E. coli* Excretion with PCB-Stress; o *E. coli* Excretion with No Stress.)	3A-7
3A-3	Survival of Total Viable Bacteria (TVC) and *Salmonella Typhimurium* in Aquarium Water Under Stressed and Unstressed Conditions. (▲ *Salmonella* with PCB Stress; △ *Salmonella* with No Stress; ● TVC, with PCB Stress; o TVC With No Stress; Oysters Removed at Day 12.)	3A-9
3A-4	Accumulation of Total Viable Bacteria (TVC) and *Salmonella Typhimurium* by the Oyster, *Crassostrea Virginica* Following PCB Stress (● TVC with PCB Stress; o TVC with No Stress; ▲ *Salmonella* with PCB Stress; △ *Salmonella* with No Stress.)	3A-10
3B-1	Chesapeake Bay Stations Sampled in the Study of March—June, 1975	3B-3
3B-2	Procedure for Enumeration and Identification of *Salmonella* Employing Non-Selective Enrichment	3B-4

LIST OF TABLES

Table		Page
3-1	Identification of Organisms Isolated in the Coliform MPN Determination Procedures	3-3
3-2	Identification of Organisms Isolated from Fecal Streptococcus MPN Determinations	3-5
3-3	Enumeration of *Vibrio Parahaemolyticus*	3-6
3-4	Detection of *Salmonella* Species	3-10
3-5	Detection of *Clostridium Botulinum*	3-11
3-6	Distribution of Micronutrients at Five Upper Bay Stations	3-15
3-7	FC/FS Ratios Calculated from Samples Collected at the Upper Bay Sampling Stations	3-25
3-8	Association of Bacteria with Particulates	3-26
3-9	Comparative Bacteriology of Oysters and Bottom Water Collected at Tolly Bar, Station 11A	3-31
3-10	Concentration of PCB-Metabolizing Bacteria in Selected Esturary and Marine Samples	3-32
3-11	Isolation of PCB-Metabolizing Bacteria from Upper Bay Stations	3-33
3-12	Concentration of Humic Acid in Upper Chesapeake Bay	3-38
3A-1	Experimental Outline for Assay of Enteric Bacteria Accumulated by the Oyster, *Crassostrea Virginica,* Following Acute PCB Stress	3A-5
3A-2	Excretion of Total Viable Bacteria (TVC) from Oysters Following PCB-Stress and *E. coli* Dosing	3A-5
3A-3	Accumulation of *Salmonella Typhimurium* in Oyster Tissue Following PCB Stress	3A-11
3A-4	Accumulation of Total Viable Bacteria (TVC) in Oyster Tissue Following PCB Stress and *Salmonella* Dosing	3A-11
3B-1	Recovery of *Salmonella* from Chesapeake Bay Samples Employing Non-Selective and Selective Enrichment	3B-7
3B-2	Physical, Chemical, and Microbiological Characteristics of Chesapeake Bay Samples from Which Recovery of Confirmed *Salmonella* was Obtained	3B-8
3B-3	Recovery of *Salmonella Enteritidis* From Samples of Water and Sediment Collected at Jones Falls Station by the Non-Selective Enrichment Procedure	3B-9
3B-4	Comparative Recovery of *Salmonella* and Related Enteric Bacteria Using the Non-Selective Enrichment Procedures	3B-11
3B-5	Comparison of Biochemical Characteristics of *Salmonella Enteritidis* Isolated from Estuarine Samples with Those of Clinical Isolates	3B-12

3. MICROBIOLOGY

3.1 Introduction

The research work covered in this report was undertaken to assess the microbiology of suspended particulates, bottom sediment, and water and to examine correlations of chlorinated hydrocarbon (CHC) contamination of the upper Chesapeake Bay with the microbiological data. Primary emphasis was placed on assessing co-transportation of bacterial indicator organisms and potential pathogens with the chlorinated hydrocarbons via suspended sediment in the upper Chesapeake Bay. The research plan included four paths of inquiry, each dealing with a separate, important and not unrelated, aspect of pollution of the upper bay. The four principal tasks were:

(1) To determine the association of indicator organisms with suspended particulate matter in the water column and the potential transport of such organisms throughout the Chesapeake Bay.
(2) To assess the deposition of pathogens and indicator organisms in upper Chesapeake Bay sediment, and to determine the potential release or re-introduction of bacteria from the sediment to the water.
(3) To relate shellfish levels of accumulated bacteria to standard or generally accepted indices of bacterial contamination (i.e., indicator organisms and pathogens).
(4) To assess the association of bacterial populations with chlorinated hydrocarbons in the upper Chesapeake Bay.

The research work, thus, was designed to provide useful information dealing with these tasks in the contractually limited time allowed for the data accumulation and analysis.

3.2 Media and Methods

3.2.1 Total Viable Counts

The total plate count agar medium used was a marine salt water-yeast extract (MSWYE) medium prepared with dilute three-salts solution(6.66‰)and used routinely in the University of Maryland laboratory for culturing estuarine bacteria. Ingredients (per liter) for the Upper Bay Extract (UBYE) agar were:

NaCl	5.0	gm
KCl	0.16	gm
$MgSO_4 \bullet 7H_2O$	1.5	gm
Difco Bacto-yeast Extract	1.0	gm
Difco Proteose Peptone	1.0	gm

The pH was adjusted to 7.2; 20 gm agar was added, and the extract was sterilized by heating to 121°C at 15 psi for 15 min.

Appropriate dilutions of water, sediment, and suspended sediment samples were spread on UBYE agar and incubated at 15°C for four weeks. Counts were made at one, two, three, and four weeks.

Preparation of dilutions- Serial dilutions of samples for TVC's were made in sterile Upper Bay (UB) salts (i.e., the salt solution used in preparing UBYE agar). Initial one-tenth dilutions of sediment were prepared by adding portions of sediment to a sterile polyethylene bottle containing 90 ml of UB salts and calibrated to a volume of 100 ml. Sediment TVC's were expressed in units of TVC/ml. Dilutions of sediment for determination of total coliform and fecal streptococcus MPN's* were prepared by diluting the one-tenth UB salts dilution one tenth in sterile 0.5 percent Difco Proteose Peptone. Suspended sediment samples were diluted directly into Difco Proteose Peptone solution for the same purpose.

3.2.2 Most Probable Number of Selected Indicator Organisms

Total coliforms - Bacto-Lactose Broth (Difco)** and other media appropriate for estimating presence of coliforms were prepared and used according to American Public Health Association (APHA) regulations. Five tube replicates of a minimum of three serial ten-fold dilutions were used. Tubes showing growth and gas formation after 48 hours at 35°C were used to inoculate tubes of Bacto-Brilliant Green Lactose Bile (BGLB) Broth (Difco). The presence of growth and gas formation in the latter medium constituted a confirmed total coliform test. Total coliform MPN values reported and listed in the data bank are confirmed values.

Fecal coliforms - Tubes of Bacto-EC Broth (Difco) were inoculated from positive lactose broth tubes and placed in a 44.5°C constant temperature water bath (with <0.5°C temperature variation) within one-half hour of inoculation after incubation for 24 hours, tubes showing growth and gas formation were recorded. IMViC*** tests were run on all cultures, that is, on both gas-producing and non-gas-producing cultures and cultures from lactose and BGLB broths after transfer to and isolation from Eosin Methylene Blue (EMB) agar (Table 3-1).

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* Most Probable Number.

** Difco Laboratories, Detroit, Michigan.

*** Gram negative asporogenous rods fermenting lactose; indole (+), methyl red (+), acetion (—), and citrate (—), typically of human fecal origin.

TABLE 3-1. IDENTIFICATION OF ORGANISMS ISOLATED IN THE COLIFORM MPN DETERMINATION PROCEDURES

Growth Response*			Total No.	No. *E. Coli*	Percent *E. Coli*
Lactose	BGLB	EC	Cultures**	Type I***	Type I
+	–	–	24	4	16.7
+	+	–	17****	1	5.9
+	+	+	81	64	79.0
+	+	(+)	2	1	50.0

* A positive response is growth and gas production. (+) indicates growth without gas production. Lactose = lactose broth, BGLB = brilliant green lactose bile broth, and EC = EC broth.

** Isolated from colonies appearing on EMB agar which had been streaked onto the EMB agar from positive MPN tubes.

*** Gram negative asporogenous rods fermenting lactose; indole (+), methyl red (–), and citrate (–), typically of human fecal origin (IMViC).

**** Intermediate chemotypes (– + – +).

Fecal Streptococcus MPN - Presumptive MPN's were obtained following APHA regulations by inoculation of BioQuest* Azide Dextrose (AD) Broth. Three 5-ml tube replicates of a minimum of three serial ten-fold dilutions were used. Inoculum volumes were multiples of five milliliters. Sample volumes greater than five milliliters were filtered through 47-mm sterile 0.45 micron Millipore filters which were rolled up and placed in single strength broth. Tubes showing growth after 48 hours at 35°C were inoculated into 10 ml of Ethyl Violet Azide (EV) Broth with a special triple loop. After incubation at 35°C for 48 hours, tubes of EV broth showing turbidity and a button of purple sediment were recorded as positive for the presence of confirmed fecal streptococcus. Fecal streptococcus MPN values reported and listed in the data bank are confirmed values. Subsequent testing of isolates from EV broth indicated that the majority of these organisms should be more appropriately labelled Enterococci, i.e., of probable human origin. (Table 3-2).

For further testing, aliquots taken from positive EV broth cultures were streaked on M-Enterococcus Agar (BioQuest). Small pink to red colonies appearing after incubation at 35°C were sub-cultured in Brain Heart Infusion Broth (Difco) and tested further after purification. Each isolate was tested for a Gram reaction, morphology, catalase, hippurate hydrolysis, starch hydrolysis, and growth at pH 9.6 and in 6.5 percent NaCl. The following scheme was used for the presumptive separation of enterococci from fecal streptococci of animal origin:

	Hippurate	Starch	Growth at pH 9.6	Growth at 6.5% NaCl
Enterococci of human origin	-	-	+	+
Other fecal streptococci	+	+	-	-

Hippurate tests were performed according to the method of Facklam, et al. (1974). Starch hydrolysis was tested using 0.2 percent soluble starch incorporated in a Nutrient Agar (Difco) overlay, which was flooded with Gram's Iodine Solution after visible growth of the cultures. Growth at pH 9.6 and 6.5 percent NaCl was tested using nutrient broth as a base. The broth was buffered with 0.05M Na_2CO_3 for the former test.

3.2.3 Isolation of Pathogenic Bacteria

Vibrio parahaemolyticus -Three methods for enumeration of *V. parahaemolyticus* were employed. One method was a direct plate procedure involving placing filters on TCBS Agar after filtration of known volumes of the sample and incubating the plates at 25°C. Dark green colonies were recorded as *V. parahaemolyticus*-like organisms (VPLO). The other methods involved an MPN procedure, using a three-tube and three-dilution series of Salt Colistin Broth or Salt Water Yeast Extract (SWYE) broth enrichments. Following incubation of the tubes at 25°C for 24 to 48 hours, the tubes showing growth were streaked onto TCBS Agar to test for the presence of VPLO (Table 3-3). The latter two methods were discontinued because of the difficulty encountered in obtaining the Salt Colistin Broth medium from commercial sources and the insufficient selectivity of the SWYE broth. Glucose-Salt-Teepol broth enrichment has been recommended by the Food and Drug Administration (FDA) and should be used in future experiments.

VPLO cultures were purified and a battery of tests, suggested by Sakazaki and cited by Kaneko (T. Kaneko, Ph. D. Thesis, 1973, p. 89), for the presumptive identification of *V. parahaemolyticus* were used. These included Gram stain (—), growth in Peptone Water containing 3 percent NaCl (+), cytochrome oxidase (+), fermentation of glucose (TSI and MOF +), gas from glucose (TSI and MOF —), acid from sucrose (TSI —), and from lactose (TSI —), Acetoin (—), H_2S production (TSI —), and growth at 43°C in SWYE (+).

*BioQuest Laboratories, Division of Becton-Dickenson, Cockeysville, Maryland.

TABLE 3-2. IDENTIFICATION OF ORGANISMS ISOLATED FROM FECAL STREPTOCOCCUS MPN DETERMINATIONS

	Source*	Total No. Cultures	Catalase (+)	Percent** Growth 6.5% NaCl	Growth pH 9.6	Starch Hydrolysis	Hippurate Hydrolysis
I.	Direct plating on M Enterococcus agar	14	7.14	92.85	78.57	n.d.***	7.14
II.	Ethyl violet azide broth	22	31.82	77.27	86.36	n.d.	13.64
III.	Ethyl violet azide broth to M Enterococcus agar	123	10.60	86.90	83.50	0.02	0.01

* Two methods for the isolation of fecal Streptococci were used: direct plating on M Enterococcus agar (I) or the azide dextrose – ethyl violet azide broth MPN sequence (II and III). The later two procedures differed in the way cultures were isolated from ethyl violet (+) tubes. In II, cultures were isolated by streaking onto a non-selective agar medium, whereas, in III, M Enterococcus agar was inoculated by streak plate procedure.

** Fecal Streptococci of human origin (enterococci) typically grow in 6.5% NaC1 and pH 9.6 broths, but do not hydrolyze starch or hippuric acid.

*** Not determined.

TABLE 3-3. ENUMERATION OF VIBRIO PARAHAEMOLYTICUS

Date	Station	Sample	Total No. VLPO Cultures*	No. Positive *V. Parahaemolyticus***
12/73***	11A	Water	7	0
	11A	Bottom sediment	3	1
	11A	Suspended sediment	5	0
	10B	Water	7	0
	10B	Bottom sediment	1	0
	5A	Water	7	1
	5A	Bottom sediment	6	1
	1A	Water	0	0
	1A	Bottom sediment	2	0
	1A	Suspended sediment	5	1
3/74†	11A	Water	5	1
	11A	Bottom sediment	3	0
	11A	Suspended sediment	0	0
	10B	Water	5	0
	10B	Bottom sediment	1	0
	5A	Water	0	0
	5A	Bottom sediment	0	0
	1A	Water	0	0
	1A	Bottom sediment	0	0
	1A	Suspended sediment	0	0
6/74††	11A	Water	10	0
	11A	Bottom sediment	0	0
	11A	Suspended sediment	0	0
	10B	Water	2	0
	10B	Bottom sediment	0	0
	5B	Water	1	0
	5B	Bottom sediment	0	0
	1A	Water	0	0
	1A	Bottom sediment	0	0
	1A	Suspended sediment	0	0
7/74†††	11A	Water	0	0
	11A	Bottom sediment	0	0
	11A	Suspended sediment	0	0
	10B	Water	0	0
	10B	Bottom sediment	0	0

TABLE 3-3. ENUMERATION OF VIBRIO PARAHAEMOLYTICUS (Continued)

Date	Station	Sample	Total No. VPLO Cultures*	No. Positive *V. Parahaemolyticus***
7/74	5A	Water	0	0
	5A	Bottom sediment	0	0
	1A	Water	0	0
	1A	Bottom sediment	0	0
	1A	Suspended sediment	0	0
9/74	11A	Water	5	3
	11A	Bottom sediment	6	2
	11A	Suspended sediment	0	0
	10B	Water	7	2
	10B	Bottom sediment	3	1
	5A	Water	1	0
	5A	Bottom sediment	0	0
	1A	Water	0	0
	1A	Bottom sediment	2	0
	1A	Suspended sediment	0	0
	Conowingo	Water	0	0
10/74†††	11A	Water	2	0
	11A	Bottom sediment	5	0
	11A	Suspended sediment	0	0
	10B	Water	4	0
	10B	Bottom sediment	0	0
	5A	Water	1	0
	5A	Bottom sediment	1	0
	Conowingo	Water	0	0
11/74†††	11A	Water	1	0
	11A	Bottom sediment	2	0
	11A	Suspended sediment	0	0
	10B	Water	0	0
	10B	Bottom sediment	0	0
	5A	Water	0	0
	5A	Bottom sediment	0	0
	Conowingo	Water	0	0
12/74†††	11A	Water	1	0
	11A	Bottom sediment	3	0
	11A	Suspended sediment	0	0

TABLE 3-3. ENUMERATION OF VIBRIO PARAHAEMOLYTICUS (Continued)

Date	Station	Sample	Total No. VPLO Cultures*	No. Positive *V. Parahaemolyticus***
12/74†††	10B	Water	0	0
	10B	Bottom sediment	1	0
	5A	Water	0	0
	5A	Bottom sediment	0	0
	1A	Water	0	0
	1A	Bottom sediment	0	0
	1A	Suspended sediment	0	0

* *Vibrio parahaemolyticus* – like organisms (VPLO) are dark green colonies on TCBS agar.

** VPLO colonies were cultured and tested to confirm their identification.

*** Salt colistin MPN enrichment procedure used. Positive tubes streaked on TCBS agar after three to four days at 25°C.

† Salt colistin MPN enrichment procedure used. Positive tubes streaked on TCBS agar after 24 hours at 25°C.

†† Salt water–yeast extract broth (SWYE) MPN enrichment procedure used. Positive tubes streaked on TCBS after 24 hours at 25°C.

††† Samples plated directly on TCBS agar and incubated at 35°C.

Salmonella - Various enrichment procedures were used for isolation of *Salmonella* spp. (Table 3-4). Selenite, selenite cystine, and tetrathionate broths were inoculated and incubated for 24 to 48 hours at 35°C or 41.5°C. Loopfuls of the enrichment were streaked on several selective differential agar media: eosin methylene blue, *Salmonella-Shigella,* brilliant green, and bismuth suflite. Later efforts to improve recovery of *Salmonella* spp. from Chesapeake Bay water samples included the use of a medium with MacConkey Agar as the base and containing 0.03 g/l novobiocin and 0.165 g/l Na_2SeO_3 to inhibit interfering gram negative bacteria. A continuous-flow, hollow fiber ultrafiltration system (Amicon Model DC 30; Amicon Corp., Lexington, Massachusetts) also was employed to concentrate large volumes of bay water to improve recovery and isolation of selected potentially pathogenic organisms from the estuarine environment.

Presumptive cultures were purified, examined for Gram stain and oxidase reactions, and inoculated sequentially into triple sugar iron agar, urea agar, and lysine iron agar. The remaining presumptive *Salmonella* cultures were subjected to further biochemical testing, following the APHA diagnostic procedure and were tested for H antigens using standard serological procedures. After one passage through a semi-solid growth medium, tube agglutination tests were run, using seven-group specific flagellar antisera (BioQuest).

Clostridium botulinum-The presence of *Cl. botulinum* was determined by indirect means by the detection of specific neurotoxin. Cooked meat medium (Difco) was prepared according to the label instructions, and a carpet tack was added before autoclaving (L. Smith, VPI Anaerobic Laboratory, personal communication). Approximately one gram of freshly collected sediment was added to the medium, which had been prepared in screw-cap tubes. A layer of mineral oil was added after inoculation, and the culture was incubated at room temperature for five to seven days, at which time the culture, or the cell-free culture supernatant solution obtained by centrifugation, was frozen at —70°C for a minimum of one day. To the supernatant solution was added one percent [(W/V)] 1:300 trypsin (BioQuest) to give a final concentration of 0.1 percent trypsin, and the mixture was incubated at 37°C for 45 minutes. For each sample, two white mice were inoculated with 0.4 ml of trypsinized supernatant. One of the pair of mice was protected by injection with *C. botulinum* polyvalent antitoxin (Communicable Disease Center, Atlanta, Georgia), i.e., by incubating a mixture of 0.4 ml supernatant and 0.1 ml antitoxin in a syringe for 30 minutes prior to inoculation. Death of the unprotected mouse within 72 hours was a presumptive positive indication of the presence of *Cl. botulinum.* A fresh aliquot of the supernatant was trypsinized, and mice were tested with and without protection, using a series of serotype specific antitoxins. When polyvalent serum failed to protect the mice, it was presumed that either the toxin titer exceeded that of the serum or a non-botulinum toxin was present. Consequently, some samples were diluted with sterile gel-phosphate (pH 6.5) buffer, and mice were inoculated with 37.5 units of bovine tetanus antitoxin prior to inoculation with the supernatant (Table 3-5).

3.2.4 Suspended Sediment Analyses

Collection of suspended sediment - Suspended sediments were harvested from fresh, aseptically collected water samples using two methods. In the first procedure, water was filtered through an eight-micron Millipore filter until clogging occurred. The filters were shaken with sterile UB salts solution* in a wide-mouth, sterile screw-cap jar to dislodge the non-filterable material. This method was abandoned after the first cruise as being cumbersome and inefficient. Suspended sediments were obtained thereafter by centrifugation. Water was placed in sterile screw-cap centrifuge tubes and centrifuged at a relative centrifugal force of 2100 g for 15 minutes. The supernatant solution as aspirated off, and the brown flocculent pellets were resuspended in sterile UB salts solution. Total viable counts of both water and pellet suspension were determined to estimate the proportion of the total viable population in the pellet.

Suspended sediment experiments - To study the association of indicator bacteria with suspended sediment, a sterile *in vitro* system was used. One-liter samples of water were placed in sterile graduated cylinders, and suspended sediments were allowed to settle for one hour. Two-hundred milliliter samples were aspirated through sterile tubing to sterile vacuum flasks. Total viable count, fecal coliform, and fecal streptococci MPN's were determined in each fraction to construct a distribution of these bacteria with the particulate fractions.

* UB salts = NaCl, 5 g; KCL, 0.16 g; $MgSO_4 \cdot 7H_2O$, 1.5g; H_2O, 1000 g.

TABLE 3-4. DETECTION OF SALMONELLA SPECIES*

Date	Station	Sample	Isolation Method**	*Salmonella* Presumptive***	*Salmonella* Positive	Fecal coliform MPN†
12/73	11A	Water	1	3	0	7.8
	11A	Bottom sediment	1	1	0	1.8
	11A	Suspended sediment	1	2	0	20.8
	10B	Water	1	4	0	2.8
	10B	Bottom sediment	1	1	0	1.8
	1A	Water	1	6	0	1600.0
	1A	Bottom sediment	1	11	0	14.0
	1A	Suspended sediment	1	13	1††	9200.0
3/74	11A	Water	2	1	1††	1.8
	11A	Bottom sediment	2	2	0	2.0
	1A	Water	2	1	0	78.0
10/74	11A	Dialysis concentrate	4	4	2††	680.0
11/74	5A	Water	4	1	0	2.0
	5A	Bottom sediment	4	2	0	1.8
12/74	1A	Water	4	1	0	33.0

* Only *Salmonella* presumptive stations reported, other stations yielded samples negative for presumptive test results.

** Method 1. Selenite enrichment streaked to EMB, deoxycholate, SS, and BG agars (35°C).
2. Selenite cystine or tetrathionate enrichment streaked to BG or Bismuth Sulfite agars (35°C).
3. Tetrathionate (41.5°C) enrichment streaked to BG or Bismuth Sulfite (41.5°C).
4. Selenite cystine (42°C) enrichment streaked to MacConkey-Selenite agar (42°C).

*** Cytochrome oxidase (–), urease (–) cultures showing red slant (alkaline) and yellow butt (acid) on TSI agar.

† Water, MPN/100 ml; sediment, MPN/ml; suspended sediment, MPN/100 ml.

†† Biochemically positive *Salmonella*, but not agglutinated by polyvalent O or H *Salmonella* antisera.

TABLE 3-5. DETECTION OF CLOSTRIDIUM BOTULINUM

Date	Station	Sample	Test For *Cl. Botulinum* Toxin	Type
3/74	11A	Sediment	–	
	10B	Sediment	–	
	5A	Sediment	–	
	1A	Sediment	+	E
5/74	11A	Sediment	–	
	10B	Sediment	–	
	5A	Sediment	–	
	1A	Sediment	–	
6/74	11A	Sediment	+	n.d.*
	10B	Sediment	–	
	5A	Sediment	–	
	1A	Sediment	+	B
	**	Menhaden	+	n.d.***
7/74	11A	Sediment	–	
	10B	Sediment	–	
	5A	Sediment	–	
	1A	Sediment	–	
9/74	11A	Sediment	–	
	10B	Sediment	–	
	5A	Sediment	–	
	1A	Sediment	–	
10/74	11A	Sediment	–	
	11A	Oysters	–	
	10B	Sediment	–	
	5A	Sediment	–	
11/74	11A	Sediment	–	
	11A	Oysters	–	
	10A	Sediment	–	
	5A	Sediment	–	
12/74	11A	Sediment	–	
	10B	Sediment	–	
	5A	Sediment	–	
	1A	Sediment	–	

* Type not determined. Mixture of B and E or other toxins present. Tetanus toxin also present.

** Dead fish specimen collected from area of fish kill in the vicinity of the Bay Bridge. Hemorrhage above eyes observed.

*** Type not determined. Tetanus toxin present.

Microscopy of suspended sediment - Direct microscopic evidence for the association of bacteria with suspended sediments was obtained by observing particles of detritus on membrane filters. The procedure for fixing and staining the specimens was essentially that of Jannasch and Pritchard (1972). Formalin was added to the suspended sediment sample to give a final concentration of 1.6 percent. Several dilutions of the sample were prepared to give optimal densities on the filters. The samples were filtered through 0.45-micron Millipore filters, and the filters were stained with Loeffler's methylene blue. An evenness in the staining was obtained by placing the filters on stain-soaked filter papers. The membrane filters were decolorized to provide contrast between cells and background by soaking the filters in several changes of distilled water. The filters were dried and placed on microscope slides. A drop of immersion oil was added on top of the filter and below, rendering the filter transparent to light. The specimens were observed by either bright-field or phase-contrast microscopy (Figures 3-1 through 3-5).

3.2.5 Humic Acid Determination

Humic substances are known to sequester heavy metals, pesticides, and other organic compounds. For this reason it was decided that the Upper Bay Survey samples should be surveyed for humic content to delineate a possible role of humic acid in transport of pesticides, nutrients, and possibly, bacteria. Humic acids were isolated and assayed according to the methods of Hair and Basset (1973) and Zitko et al. (1973). Dissolved, particulate, and sediment humic acid concentrations were measured spectrophotometrically, after extraction and purification. A curve was constructed showing optical density at 250 nm* plotted against concentration of freshly prepared humic acid standard in 0.5 N NaOH. Humic acid standard was isolated from farm soil following the method described by Hair and Basset (1973).

3.2.6 Nutrient Analyses

Samples of water, freshly collected using Niskin sterile bag samplers, were filtered through Whatman GF/c filters into a vacuum flask. The flask was rinsed with the initial 50 ml of filtrate, which was then discarded prior to filtration of the remaining sample. Approximately 250 ml of filtrate was transferred to 300 ml 6 N HCl-washed bottles fitted with polyethylene-lined caps. These samples were frozen aboard ship and later stored in the laboratory at —70°C. Analyses were performed at the Chesapeake Bay Institute of The Johns Hopkins University, essentially according to the methods described by Strickland and Parsons (1972). (See Table 3-6).

Total dissolved phosphorus and nitrogen were obtained from samples treated by irradiation with ultraviolet light.

3.2.7 PCB-Metabolizing Bacteria

Isolation of polychlorinated biphenyl-resistant bacteria was accomplished as the research progressed. Several species of bacteria have been shown by other workers to be resistant to, or unaffected by, high concentrations of polychlorinated biphenyls (PCB's) and DDT (Greer and Keil, 1974). In addition, an *Achromobacter* sp. has been shown to metabolize actively several PCB's of various chlorine content (Ahmed and Focht, 1973). In the work reported here, bacteria resistant to high concentrations of PCB, or capable of metabolizing PCB, were isolated from various samples using a combined enrichment and plating procedure. The polychlorinated biphenyl, Aroclor® 1254 (Monsanto Co., St. Louis, Missouri) was dissolved in acetone and coated on a support of Celite® (J. T. Baker Chemical Co., Philipsburg, New Jersey). Appropriate quantities of coated celite were added to a basal salts broth and autoclaved. Dilutions of sediment and water were added to the primary enrichment broth and incubated at 25°C for two weeks. Samples of each enrichment broth were then plated on a solid medium containing 500 mg/1 PCB 1254 as the sole carbon source. Additional water samples were plated directly on PCB 1254 agar plates in an effort to determine the quantitative levels of PCB 1254-metabolizing bacteria present at each of the Chesapeake Bay stations routinely sampled in this study.

* nm is nanometers or 10^{-9} meters

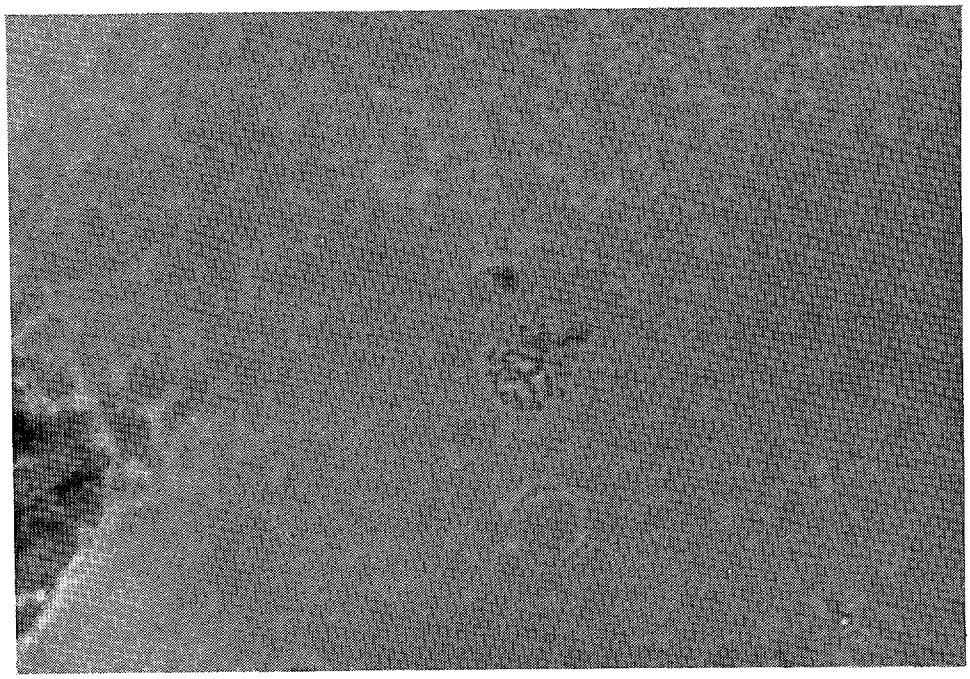

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Figure 3-1. Bacterial Association with Particulates (Light Microscopy 1,000 X)

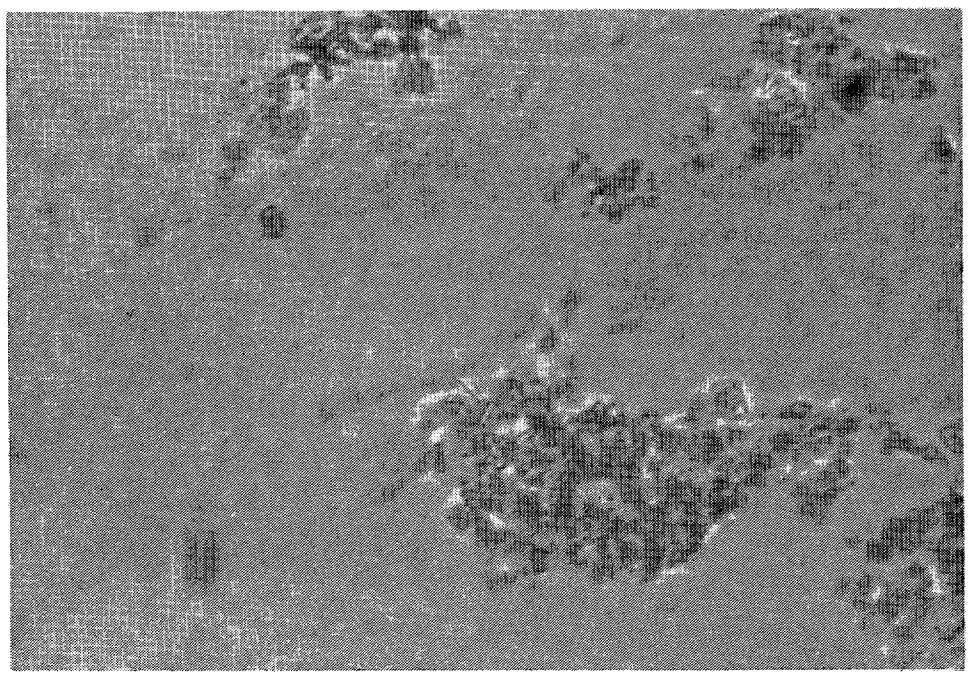

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Figure 3-2. Bacterial Association with Particulates (Light Microscopy 1,000 X)

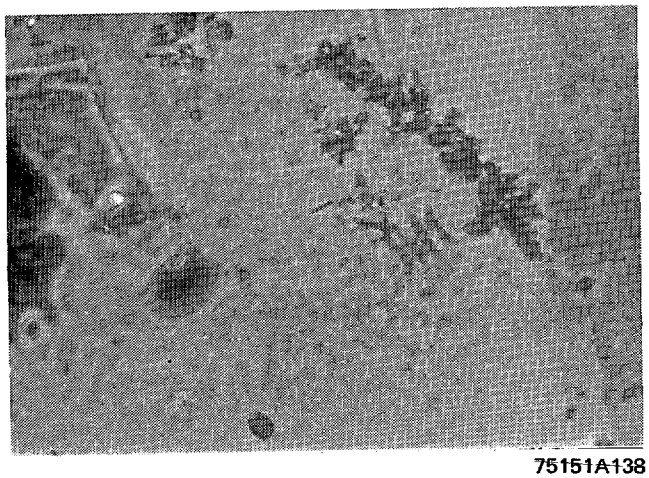

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Figure 3-3. Bacterial Association with Particulates (Light Microscopy 1,000 X)

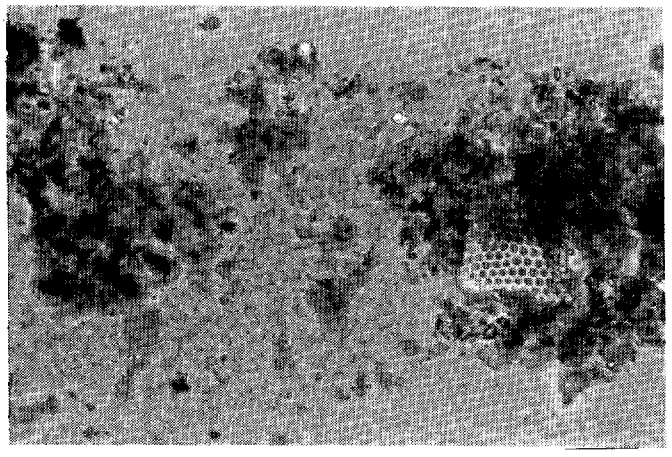

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Figure 3-4. Bacterial Association with Particulates (Light Microscopy 1,000 X)

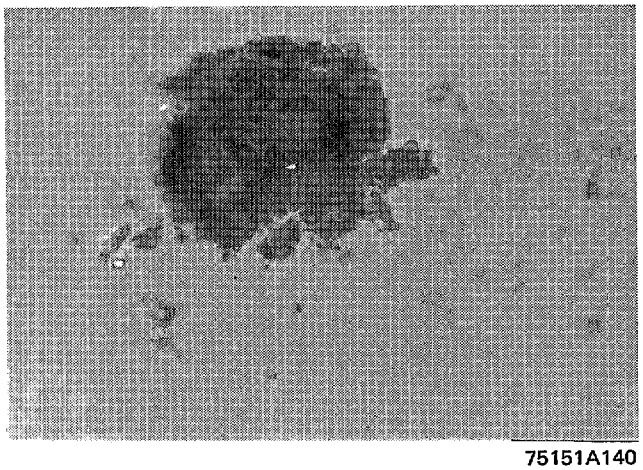

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Figure 3-5. Bacterial Association with Particulates (Light Microscopy 1,000 X)

TABLE 3-6. DISTRIBUTION OF MICRONUTRIENTS AT FIVE UPPER BAY STATIONS

Station	Nutrient	μMoles					
		July	Sept	Oct	Nov	Dec	Mean
Conowingo	$P-PO_4^{-3}$	-	0.7	0.5	0.9	-	0.7
	$NO_2^{-1}+NO_3^{-2}$	-	40.0	50.0	50.0	-	47.0
1A	$P-PO_4^{-3}$	0.1	0.5	-	-	-	0.3
	$NO_2^{-1}+NO_3^{-2}$						
5A	$P-PO_4^{-3}$	0.4	0.7	0.5	0.5	0.2	0.5
	$NO_2^{-1}+NO_3^{-2}$	45.0	35.0	25.0	40.0	25.0	34.0
10B	$P-PO_4^{-3}$	0.1	1.7	1.1	0.8	0.6	0.9
	$NO_2^{-1}+NO_3^{-2}$	<5.0	25.0	<5.0	10.0	5.0	10.0
11A	$P-PO_4^{-3}$	0.2	0.5	0.3	0.7	0.3	0.4
	$NO_2^{-1}+NO_3^{-2}$	15.0	15.0	25.0	20.0	85.0	32.0

The PCB 1254 agar plates were incubated at 15°C for two weeks. Individual bacterial colonies were isolated and tentatively classified to generic level. Pure cultures of PCB-resistant or metabolizing bacteria were then employed in respiration studies to determine the effect of various concentrations of PCB 1254 on respiratory metabolism.

Respiration studies of PCB resistant bacteria - Effects of PCB on respiration were determined using a Gilson respirometer, Model GRP 20 (Gilson Medical Electronics, Middleton, Wisconsin). Bacteria used in the respiration studies were adapted to PCB 1254 by incubating cultures in logarithmic phase of growth in a basal broth containing 1.0 g/l PCB 1254. Transfers of the PCB adapted cultures were made in fresh media containing no PCB 1254, incubated for appropriate time intervals, centrifuged, washed with sterile phosphate buffered saline, and added to sterile respirometer flasks containing various concentrations of sterile PCB 1254 coated on celite. Oxygen consumption was monitored for several hours and was compared with rates of oxygen consumption of control bacterial cultures incubated with untreated diatomaceous earth.

A [U—^{14}C] glucose decomposition assay (Kadota, 1972) was employed to examine the effect of PCB 1254 on a mixed marine bacterial population. Twenty gram sediment samples were placed in duplicate glass dark bottles to which an additional 100 ml of artificial sea salts were added. Another pair of dark bottles received 100 ml of surface water. Each sediment and water sample received 2.5 μCi* of [U—^{14}C] glucose (100 mg/l glucose). One of each pair of sediment and water samples was supplemented with 1.56 mg PCB 1254 on Celite (15.6 mg/1 PCB 1254). The dark bottles were stoppered, and a flow of air carried $^{14}CO_2$, (from the decomposition of [U—^{14}C] glucose) to liquid scintillation vials in which $^{14}CO_2$ was trapped in 7.5 ml of scintillation cocktail containing: 27 ml phenylethylamine, 27 ml absolute ethanol, 0.51 g Omnifluor (New England Nuclear, Boston, Massachusetts), and 100 ml toluene. The samples were flushed for an initial hour and then were allowed to incubate undisturbed for 24 hours at 20 to 25°C. At 24 hours the samples were again flushed with air for one hour, and the $^{14}CO_2$ was trapped in the scintillation cocktail. An additional 7.5 ml of a scintillation fluid containing 5.1 g Omnifluor in 1,000 ml toluene were added to the scintillation vials. The $^{14}CO_2$ samples were stored for a period of five to seven days and were counted on an Intertechnique SL40 liquid scintillation counter (Teledyne Corp., Westwood, New Jersey). A comparison of the effects of PCB 1254 on glucose decomposition was then made using a multivariate analysis of variance.

3.2.8 Bacteriological Analyses of Oysters

Benthic filter feeders can effectively concentrate and retain various pathogenic bacteria (Janssen, 1973) and viruses (Metcalf and Stiles, 1965). The oyster, *Crassostrea virginica*, in the Chesapeake Bay may concentrate and/or retain fecal indicator organisms or potential pathogens in the areas where fecal contamination poses a hazard, (viz., regions of the upper Chesapeake Bay). To determine the extent of this problem, oysters were collected from Tolly Bar near Station 11A and were cleaned and shucked immediately upon sampling. Using procedures prescribed by APHA (American Public Health Association, 1970), homogenized oyster tissues were assayed for total and fecal coliforms, fecal streptococci, Salmonellae, VPLO, Clostridia and total viable, aerobic, heterotrophic bacterial count. All isolation procedures were as described (*vide supra*) in analyses of water, sediment, and suspended sediment samples.

3.3 Results and Discussion

3.3.1 Total Viable Counts

Total viable bacterial counts (TVC) can be interpreted to reflect input of microorganisms from extra-aquatic sources. They may also be employed to described the trophic conditions of a given habitat, i.e., availability of growth-supporting organic matter and micronutrients. Fluctuations in TVC between sampling stations observed in this study may be a result of either or both conditions.

* μCi is microcuries

As shown in Figures 3-6 through 3-9 and as can be seen from the data recorded in Volume III, there was a bimodal distribution in total viable counts for bottom water samples for all stations sampled. The highest TVC's were obtained during the winter and spring months. The lowest TVC's were recorded for the months of July, September, and October. Although not as marked as in the case of the bottom water, the TVC's of the sediments at Station 5A, 10B, and 11A followed essentially the same distribution (Figure 3-8). TVC's of the sediment at Station 1A followed an anti-coincidental distribution, when compared with the other samples (i.e., peak TVC's occurred during June, July, and September, with low TVC's occurring in Station 1A sediment in the winter and early spring). The highest recorded counts over the year were obtained at Station 1A, about 10 to 100 times greater than those at Stations 5A and 10B. In only three instances, in the case of the March water and sediment samples and May water samples, were counts obtained at Station 11A higher than those at Station 1A. A comparison of total viable counts for water samples collected at Station 1A and at Conowingo Dam (Figure 3-6) indicated a slight influx of organisms from the Susquehanna River at Station 1A. Total viable counts at Conowingo Dam exceeded total viable counts at Station1A by 50 percent in September, while all other recorded values for Conowingo water were less than or equal to the values for Station 1A water.

3.3.2 Most Probable Number Index of Indicator Organisms

MPN levels of total coliforms, fecal coliforms, and fecal streptococci fluctuated seasonally and with distance from Station 1A to 11A (longitudinally) in samples of Chesapeake Bay water examined in this study (See Figure 3-7). In general, MPN values decreased from winter to summer, with a gradual increase in the number of indicator organisms in the fall. The MPN trend for Station 11A, however, was characteristically higher in the summer months, June through September. In most cases, total coliform levels (representing both human and non-human sources of coliforms) were higher than fecal coliforms and fecal streptococci, as would have been expected. Fecal streptococci in bottom water closely paralleled fecal coliform trends except at Station 11A where fecal streptococci rose dramatically during the summer months, whereas fecal coliforms were low. Highest MPN values were obtained for water samples from Station 1A in all cases. The entry of total coliforms to the upper Chesapeake Bay via the Susquehanna River appears to be significant, judging from the high total coliform MPN values measured at Conowingo Dam. Fecal coliform and fecal streptococci levels at Conowingo Dam were relatively high, indicating superimposition of these fecal indicators at Station 1A. The sharp peaks in total coliforms and fecal coliforms in December 1974 at Station 5A most likely reflect a point source of pollution. (Unfortunately, the *R/V RIDGELY WARFIELD* sanitary holding tanks are automatically discharged when full, and this occurred just prior to arrival on station when Station 5A was sampled. Thus, one can conclude that the peak MPN observed at Station 5A attests to the responsiveness of the MPN technique to sewage input.)

It is interesting to note that the December 1974 peak in fecal coliforms in the water at Station 5A was not observed for fecal coliforms in the sediment (See Figure 3-8). It is extremely unlikely that adsorption or deposition of coliform bacteria on sediments would occur within the time frame of discharge and sampling (approximately one hour).

An observable irregularity did occur in MPN values for water and sediment (See Figures 3-7 and 3-8) at Station 1A. A substantial (100-fold) increase in total coliforms and fecal coliforms occurred in the sediments at Station 1A during the summer of 1974. Fecal streptococci levels held fairly steady in the summer months, although a 100-fold fluctuation occurred seasonly. It should be pointed out, however, that fecal streptococci levels in December 1973 and December 1974 were not significantly different. It is clear from these data that extensive sampling is required for adequate measurement of the incidence and distribution of indicator organisms (i.e., their flux within a given aquatic habitat). In general, MPN values at all of the sampling stations were lower in sediments than in the water. Also, the sediment data indicated somewhat less of a seasonal fluctuation occurring. Fecal streptococci levels were frequently higher than total coliform levels in the sediment and were, in general, higher than fecal coliform levels in the sediment.

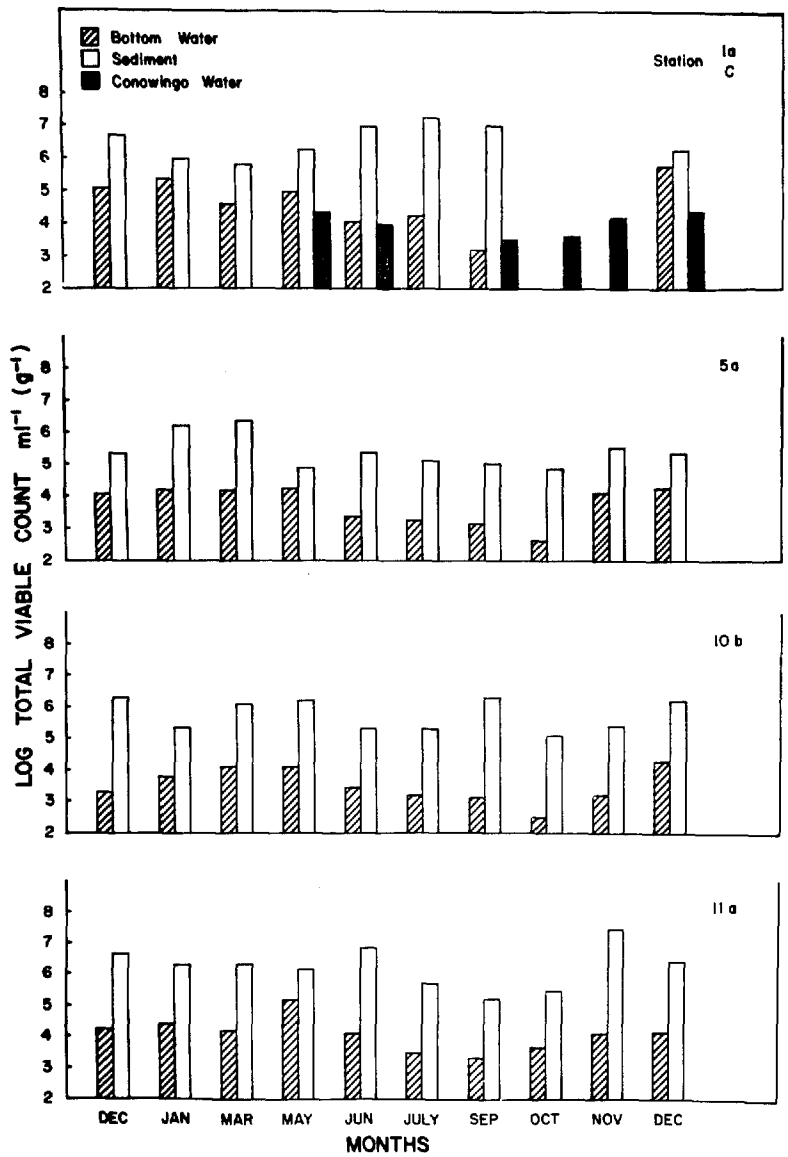

Figure 3-6. Comparison of the Temporal Distribution of Total Viable Bacteria in Water and Sediment Among Upper Bay Samples

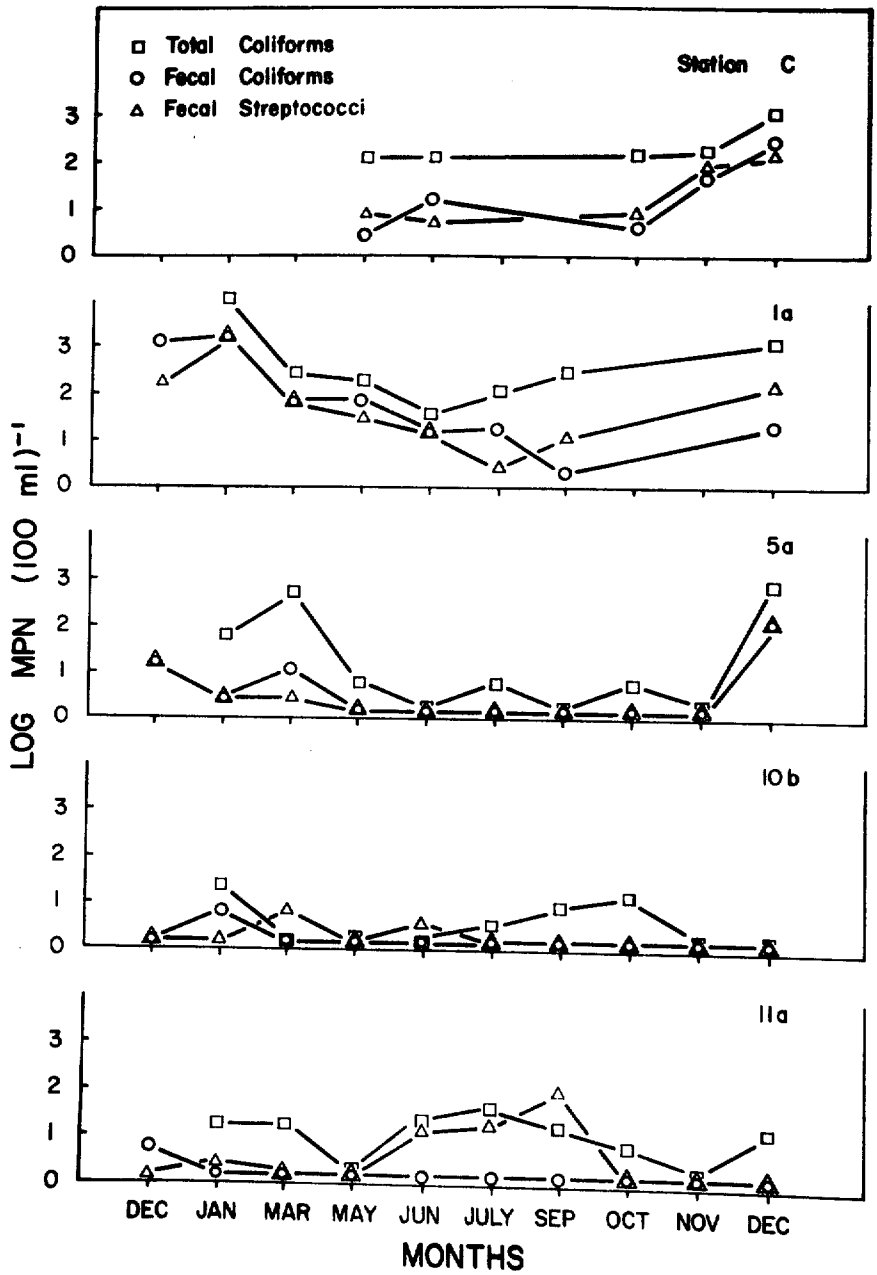

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Figure 3-7. Comparison of the Temporal Distribution of Fecal Indicator Organisms in the Bottom Water Among Upper Bay Samples

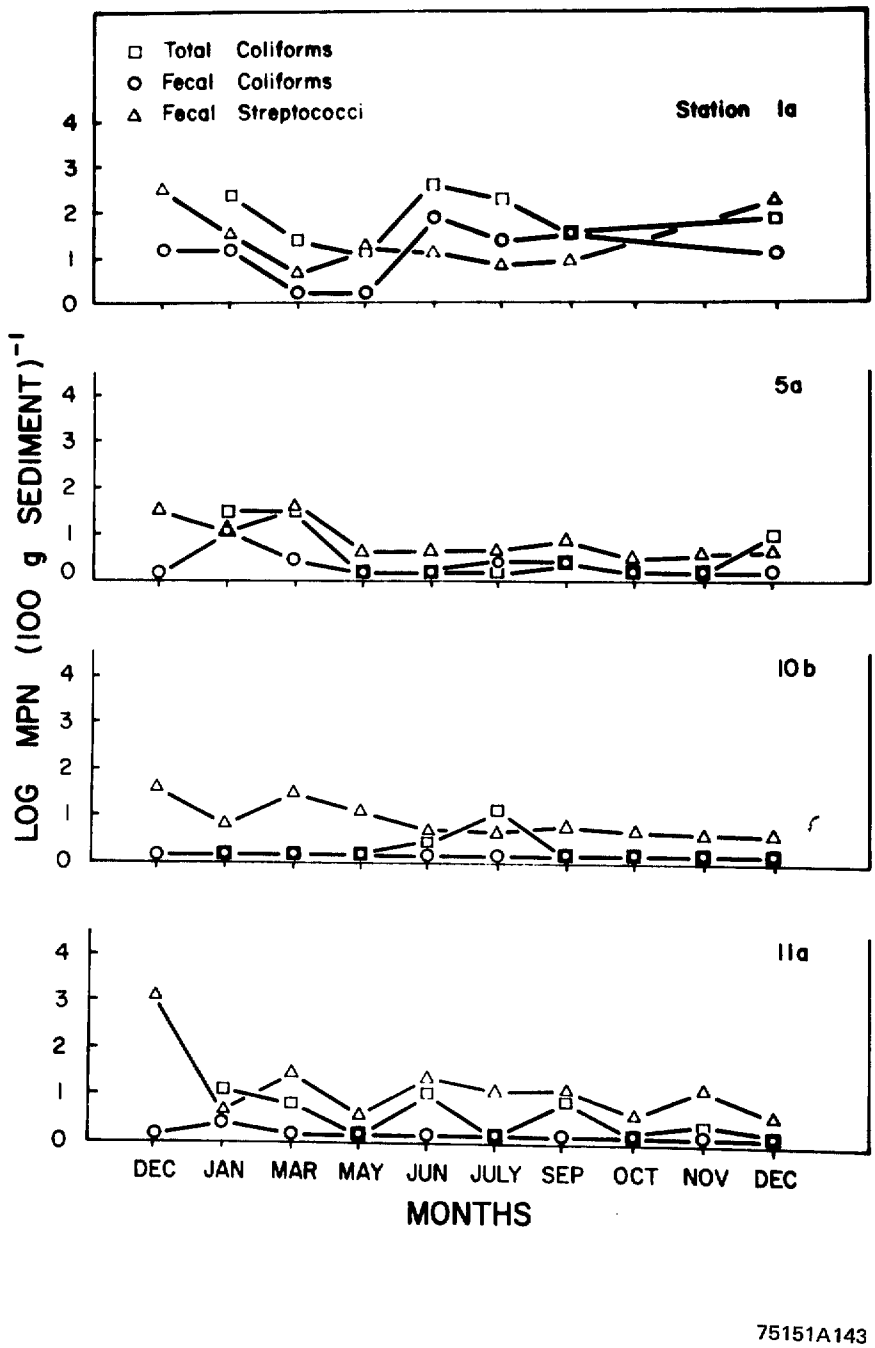

Figure 3-8. Comparison of the Temporal Distribution of Fecal Indicator Organisms in the Sediment Among Upper Bay Stations

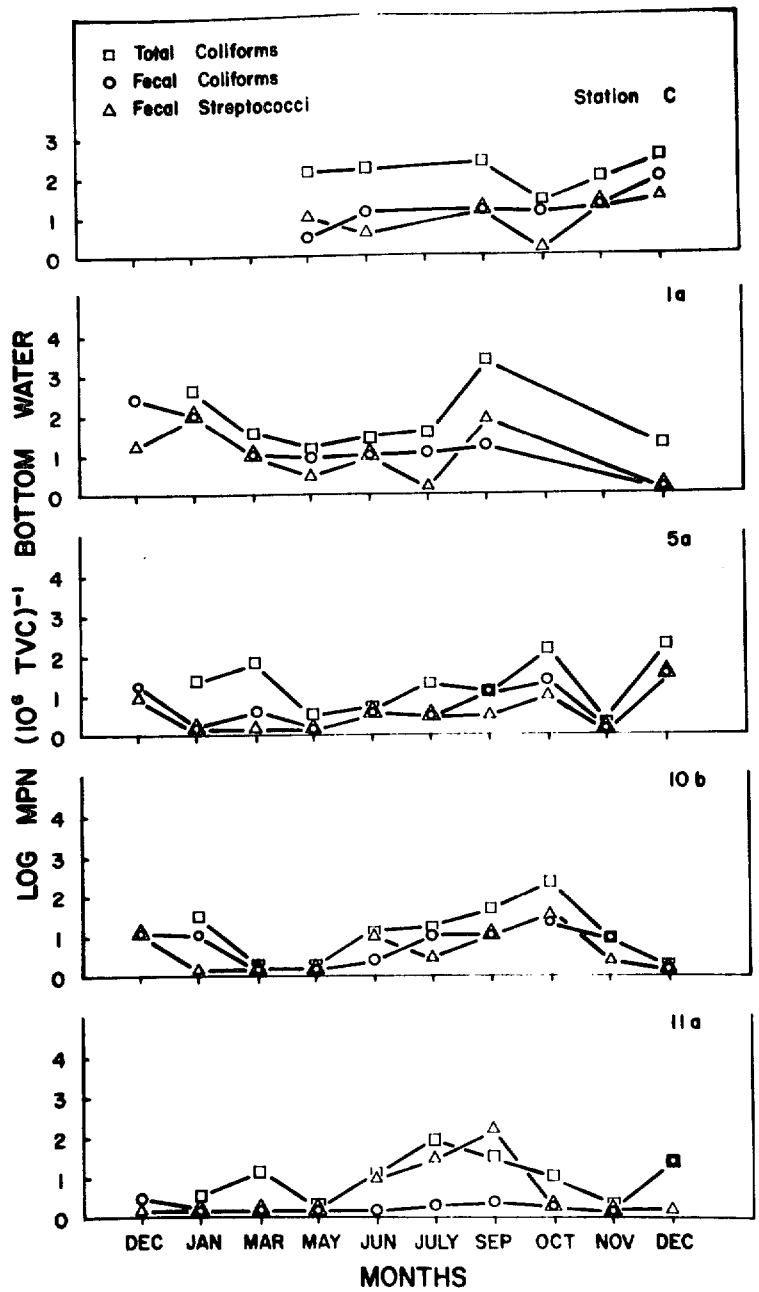

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Figure 3-9. Comparison of Waterborne Indicator Organisms in Relation to the Temporal Distribution of Total Viable Bacteria Among Upper Bay Stations

Several genralizations can be drawn from the data shown in Figures 3-6 through 3-8. Higher coliform levels detected during the winter months may result from effects of decreased water temperature on the organisms, longer survival times of certain microbial groups, and possible increased nutrient flux from decomposition of summer productivity and watershed drainage. Fecal streptococci survival appears to be enhanced in the sediments at all stations where the fecal streptococci and fecal coliform populations maintained generally low populations.

3.3.3 Efficiency of Identification of Indicator Organisms

Cultures of fecal coliforms and fecal streptococci were routinely characterized to test the accuracy of the enumeration methods used (Tables 3-1 and 3-2). Approximately 80 percent of the fecal coliforms were phenotypically *Escherichia coli* Type I, which is considered to be of human origin. Some stations produced more false positives than others, and these cultures proved to be a number of intermediate coliform chemotypes as well as non-coliform organisms. Station 10B more commonly gave false positive coliforms MPN's. This finding underlines the need to characterize MPN's for each environment beyond the presumptive level of accuracy. Fecal streptococci isolates were examined using a number of selected tests and the results indicated that more than 80 percent were enterococci. Although not exclusively of human fecal origin, the majority of these enterococci are considered to originate from the human intestinal tract.

3.3.4 FC/FS Ratios

Ratios of fecal coliforms to fecal streptococci are a relative index, describing the proportion of these fecal indicator organisms within a given population. Geldreich and Kenner (1969) have cited the ratio of fecal coliforms to fecal streptococci (FC:FS) as a characteristic for given types of pollution or sources. The ratio itself is not a quantitative indication of pollution but a qualitative pollution index. Domestic sewage yields FC:FS ratios greater than 4.0, whereas ratios less than 0.7 are interpreted as indicating warm blooded animals, other than man, as the source. Lear and Jaworski (1969) reported that their data collected for upper Potomac River estuary stations indicted FC:FS ratios in excess of 4.0. FC:FS ratios greater than 4.0 were observed at all of the sampling stations included in this study, except Station 5A. Data for Station 1A showed more (33 percent) FC:FS ratios greater than 4.0, compared with data for samples from Conowingo Dam, Station 10B, and Station 11A each of which yielded one sample (less than 16 percent of the samples) greater than 4.0. It is notable that suspended sediment samples from Station 1A on three occasions greatly exceeded the 4.0 ratio, whereas none of the suspended sediment samples from Station 11A revealed FC:FS ratios greater than 4.0. Sediment samples at Stations 5A, 10B, and 11A all yielded FC:FS ratios indicating non-human sources of contamination at or near those stations. Since differential rates of survival exist among fecal coliforms and fecal streptococci in aged samples, lower FC:FS ratios may reflect either time and/or geographical distance factors relative to the pollution source.

3.3.5 Occurrence of Indicator Organisms Relative to Total Viable Counts

In order to determine the contribution of indicator organism populations to the total viable bacterial population, indicator organism MPN per million TVC was computed (See Figure 3-9). The relative proportions of fecal coliforms and fecal streptococci in the total viable counts decreased southwards from Station 1A to 11A during the winter months. During the spring months, the proportion approached uniformity among sampling stations. However, starting in June at Station 11A, a gradual rise in the relative abundance of indicator organisms in the water column occurred (See Figure 3-9). This increase was mirrored at the other sampling stations at progressively later dates from Station 11A to Station 1A. This pattern of occurrence of indicator organisms was not reflected in the bottom sediments (Figure 3-10.) Although Station 11A water and sediments showed coincident peaks in the relative proportion of fecal coliforms and fecal streptococci making up the total viable, aerobic, heterotrophic

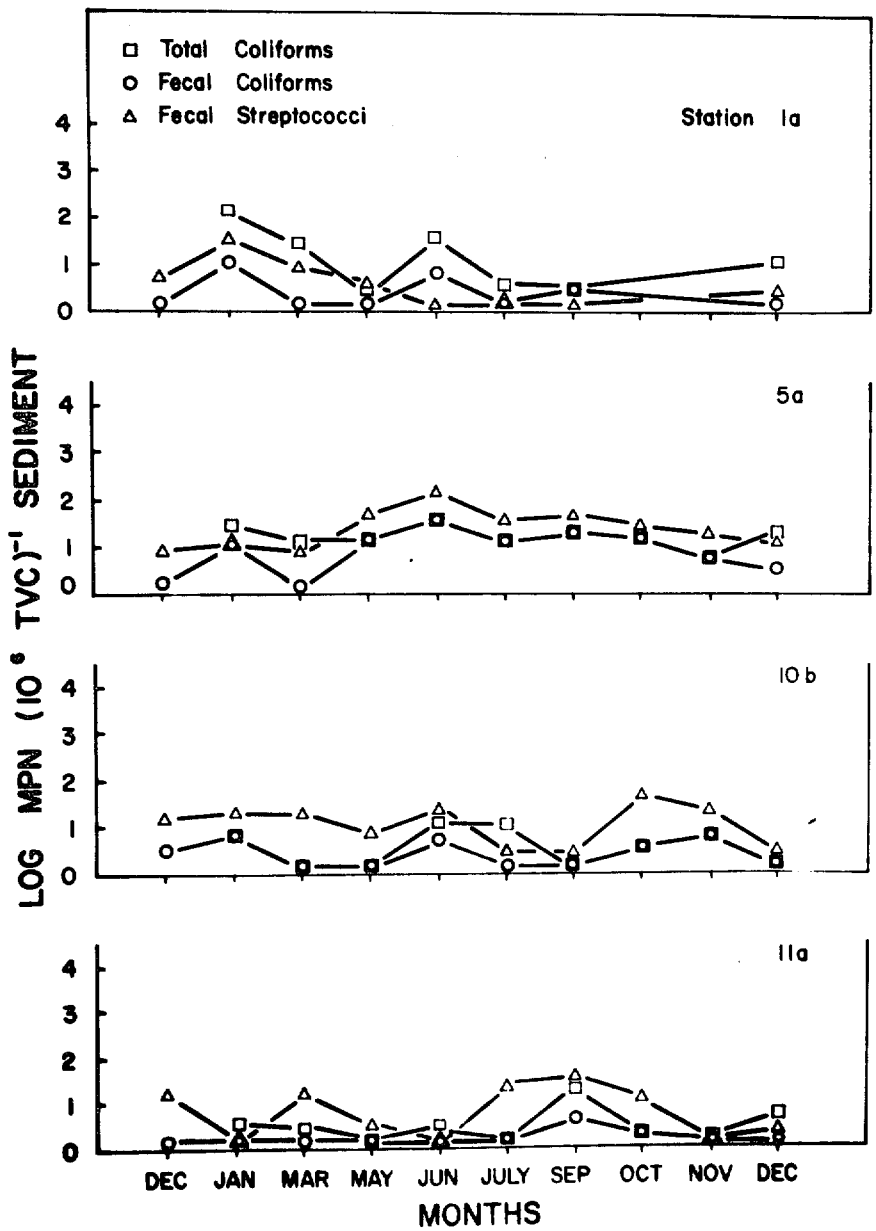

Figure 3-10. Comparison of Sediment-Bound Indicator Organisms in Relation to the Temporal Distribution of Total Viable Bacteria in Upper Bay Sediments

bacterial population, the chronological progression in proportion of indicator organisms per population did appear in the sediments at Stations 1A to 11A. Station 5A and 10B sediments showed somewhat constant proportions of indicator organisms throughout the year, whereas the abundance of fecal coliforms and fecal streptococci showed greater fluctuation in 1A sediments.

These results were found to be strongly correlated with results presented in Figures 3-7 and 3-8. It must be remembered that TVC's are almost always significantly higher in sediments as opposed to bottom water (See Figure 3-6). Thus, the relative abundance of indicator organisms would be expected to be lower in sediment samples. However, in several instances a greater proportion of fecal streptococci was observed in sediment samples collected at Station 10B and 5A. This can be interpreted as indicating enhanced survival time of fecal coliforms in sediments or to the distance from and time elapsed between the input of fecal contamination and the recovery of the fecal streptococci. It can be concluded, from the abundance of fecal indicators observed at Station1A, that a source of contamination exists in the vicinity of that station. When these data are examined with the FC:FS ratio data for Station 1A (Table 3-7) the contamination is concluded to be domestic sewage. The source of contamination at Station 1A is, in part, masked by the dilution of Chesapeake Bay water with Susquehanna River water, the latter having high total coliform levels.

The progressive increase in numbers of fecal indicator organisms occurring from summer to fall at Stations 11A through 1A could also be interpreted as being linked to increased summertime activities in Chesapeake Bay related to recreational use. However, this is completely speculative, since the pattern of peak occurrence of indicator organisms shows consistently later occurrence during the year, moving in a longitudinal direction southwards from Stations 1A to 11A.

3.3.6 Association of Bacteria with Particulates

A potential mechanism of transport for bacteria from water to sediment and vice versa is provided by suspended particulate matter. As indicated by data presented in Table 3-8, a highly significant proportion of both total viable bacteria and selected indicators of fecal pollution were found to be associated with particulate matter in the water column. Up to 53 percent of the total viable bacteria in Station 11A water samples was found to be associated with particulate matter collected in 2100 X g centrifugation pellets. TVC found to be associated with particulates in Station 1A samples exceeded 25 percent in three of five determinations. Similarly, the centrifugation studies showed a significant proportion of the total MPN of the fecal indicators at Station 1A and a majority of the fecal indicators at Station 11A were associated with the suspended particulates. An earlier study, comparing retention of TVC on 8 and 1.2-micron membrane filters, revealed that bacteria at Station 11A were predominantly associated with small sized particles than were bacteria at Station 1A. However, a sharp correlation between concentration of suspended sediment at a given station and bacteria associated with particulates was not observed.

Results of a seasonal comparison of bacteria associated with suspended matter carried out at Stations 1A and 11A (Figure 3-11) suggested both TVC's and number of indicator organisms decreased from winter to spring at Station 1A. An identical pattern in TVC distribution was observed at Station 11A. However, the distribution of indicator organisms associated with suspended sediment rose from December to January and remained stable until September, at which time the numbers of both total coliforms and of fecal coliforms associated with the particulates declined, whereas the number of fecal streptococci remained stable until December 1974. In general, counts of bacteria associated with particulates obtained for samples collected at Station 1A were higher than those at Staion 11A throughout the year.

Collectively, the above data show that total numbers of fecal coliforms and fecal streptococci vary temporally and spatially. Further, transport via association with suspended sediments is strongly indicated. It must be emphasized that the evidence accumulated in this study point out the difficulty and danger in depending on one bacterial group as an indicator of the presence of human pathogens in suspended sediment.

TABLE 3-7. FC/FS RATIOS CALCULATED FROM SAMPLES COLLECTED AT THE UPPER BAY SAMPLING STATIONS

Station	Dec.	Jan.	Mar.	May	June	July	Sept.	Oct.	Nov.	Dec.	Average
Conowingo											
Water	—	—	—	0.5	2.5	—	0.8	2.2	5.0	1.9	2.3
1A											
Water	7.3	1.1	0.9	1.8	1.4	5.0	0.3	—	—	0.2	2.2
Bottom sediment	0.03	0.4	0.3	1.0	5.4	4.5	5.8	—	—	0.6	2.3
Suspended sediment	0.04	0.1	11.3	0.3	3.0	8.2	10.8	—	—	1.8	4.4
5A											
Water	1.5	1.1	2.8	1.1	1.3	1.3	2.5	2.7	2.7	1.0	1.8
Bottom sediment	1.9	0.7	0.1	0.3	0.3	0.3	0.5	0.5	3.0	0.3	0.9
10B											
Water	2.5	1.1	11.1	1.0	1.0	0.4	3.0	0.8	3.0	1.4	2.3
Bottom sediment	0.03	0.3	0.04	0.1	0.3	0.3	1.9	0.3	0.3	0.3	0.4
11A											
Water	5.2	0.7	1.0	1.0	0.13	0.07	0.02	1.1	3.0	1.5	1.6
Bottom sediment	0.001	0.8	.04	0.3	0.6	0.1	0.1	0.3	0.08	0.3	0.2
Suspended sediment	3.3	0.6	0.3	0.3	0.5	0.3	0.2	0.3	0.004	7.5	1.5
Average	2.2	0.5	2.8	0.7	1.5	2.0	2.4	1.0	2.1	1.5	

TABLE 3-8. ASSOCIATION OF BACTERIA WITH PARTICULATES

Date	Percent of TVC* 8 Micron	Percent of TVC* 1.2 Micron	Percent of TVC* 2,100 x g Pellet	Percent of MPN** Fecal Coliform	Percent of MPN** Fecal Strep.	Suspended Sediment (mg/1)
		11A			11A	
12/73	54.0	81.8	--	--	--	5.6
1/74	30.5	63.5	17.9	89.0	<93.8	7.98
3/74	80.0	79.4	19.3	--	--	9.62
5/74	--	--	53.1	--	<83.3	10.48
6/74	--	--	11.4	28.4	7.0	3.66
7/74	--	--	--	43.8	9.9	7.38
9/74	--	--	15.9	< 78.0	74.7	6.34
10/74	--	--	3.1	< 1.4	--	
11/74	--	--	5.6	16.0	15.6	
12/74	--	--	--	--	62.5	
		1A			1A	
12/73	--	--	--	--	--	42.42
1/74	91.5	94.7	25.2	7.5	--	33.86
3/74	94.5	94.4	1.4	76.3	6.1	12.66
5/74	--	--	26.8	3.5	22.3	43.34
6/74	--	--	27.9	39.4	<18.0	13.2
7/74	--	--	0.22	93.2	<57.1	13.52
9/74	--	--	--	--	--	7.70
10/74	--	--	--	--	--	
11/74	--	--	--	--	--	
12/74	--	--	--	--	--	

* Percentage of the TVC retained by 8 and 1.2 pore size membrane filters or sedimented by centrifugation at 2,100 x g.

** Percentage of MPN indicator organisms removed by centrifugation of water at 2,100 x g.

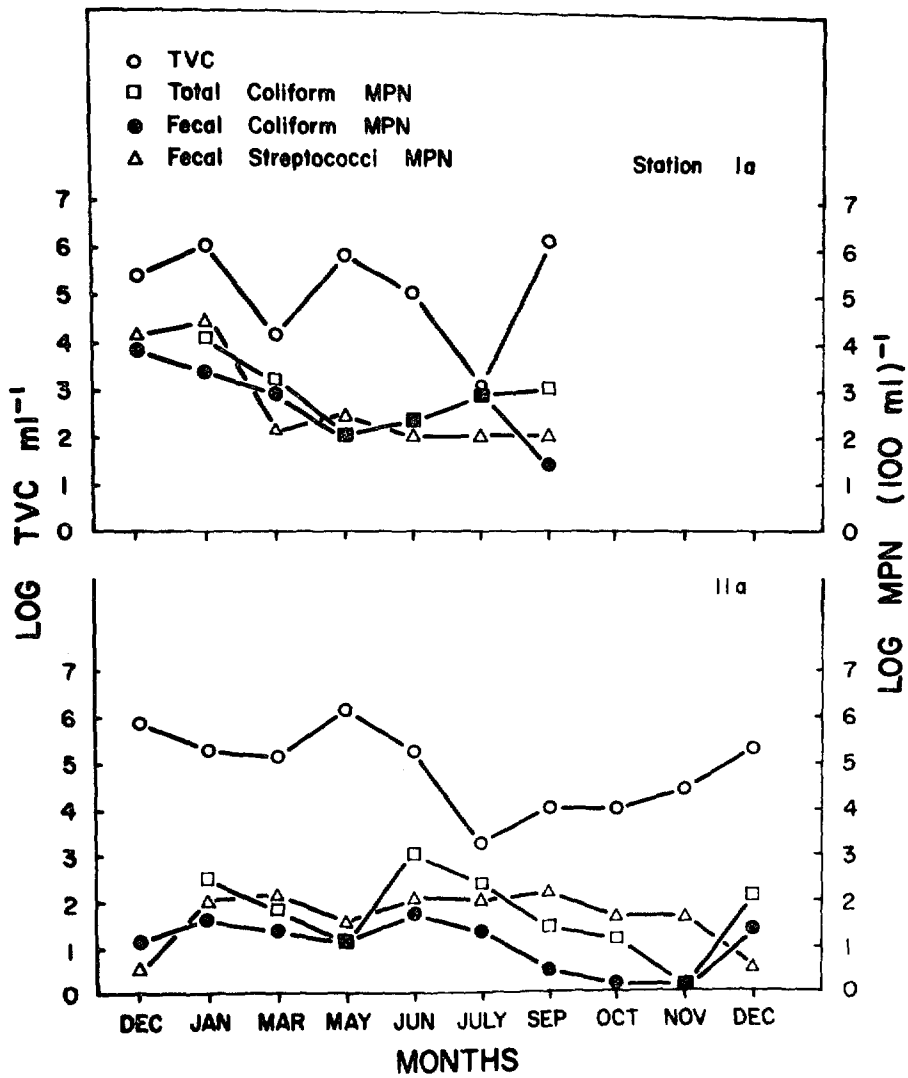

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Figure 3-11. Comparative Recovery of Bacteria Associated with the Suspended Sediment at Stations 1A and 11A

More direct evidence for the association of bacteria with suspended sediments was obtained (See Figures 3-1 through 3-5). Rod-shaped bacteria were observed to be associated with amorphous clumps of material and, less frequently, with diatoms found in suspended sediment preparations from Stations 11A and 1A. Preliminary information on the settling of indicator organisms suggests a relationship of these bacteria with debris and suspended particulates.

3.3.7 Isolation of Potentially Pathogenic Bacteria

Salmonella - The isolation of *Salmonellae* from fresh and estuarine waters has been reported by a number of investigators. Hendricks (1971) and Van Donsel and Geldreich (1971) have demonstrated that greater recoveries of *Salmonellae* can be made from sediment than from surface water samples. The occurrence of *Salmonella* in the aquatic environment may be indicative of contamination by fecal material of human and animal origin, from both treated and untreated sewage.

Of the 115 samples of water, sediment, and suspended sediment collected from the five regularly sampled stations included in this study, none have been found to contain detectable authentic *Salmonella* spp. when examined by the standard methods. (See Table 3-4.) Two isolated, tentatively identified as *Salmonella* spp. and having biochemical characteristics of *Salmonella*, were isolated. However, neither culture was identified serologically as a *Salmonella* sp. when H-antisera were employed. A number of isolates were shown to agglutinate in polyvalent salmonella-O antiserum, but they were subsequently found to be H-negative. These findings are consistent with the occurrence of cross-reacting biochemical and serological types among the Family *Enterobacteriaceae.* Among the more common isolates that were characterized to genus were *Pseudomonas* spp. and other *Enterobacteriaceae*, including coliform organisms and *Citrobacter* spp.

The low frequency of isolation of *Salmonella* in the upper Chesapeake Bay is probably a consequence of the low density or frequency of occurrence of these organisms, rather than the methods used. Van Donsel and Geldreich (1971) reported that the frequency of isolation of *Salmonella* was related to fecal coliform density, based upon similar survival rates in sediment for these two groups of microorganisms. Interestingly, the ratio of *Salmonella* isolation frequency to fecal coliform MPN in estuarine water was observed to be less than half that of fresh water, for levels of one to 200 fecal coliforms per 100 ml. The same authors indicated that *Salmonella* were not isolated very frequently from sediment collected at sites with less than 200 fecal coliforms per 100 ml of surface water, or 1,000 to 10,000 per milliliter of sediment. Similarly, Spino (1966) showed that no *Salmonella* were isolated from fresh waters with less than 220 fecal coliforms per 100 ml. Lear and Jaworski (1969) reported that no *Salmonella* were isolated from the upper Potomac River estuary, when fecal coliforms were present in numbers less than 100 per 100 ml. The data given in Table 3-5 indicate that, using the results of Lear and Jaworski (1969), few of the samples examined in this study yielded levels of fecal coliforms high enough to make isolations of *Salmonella* feasible. Van Donsel and Geldreich (1971) calculated that the average ratio of fecal coliforms to *Salmonella* in sediment was 14,000 to one. On the basis of such a calculation, the sample volumes used would have precluded detection of *Salmonella.*

Salmonella, occurring at levels not detectable by the methods initially employed in this study, nevertheless, may pose a serious public health threat. Quantities of surface water, up to 100 gallons, were concentrated using the Amincon hollow-fiber, continuous-flow dialysis concentrator. This method allowed for all particle sizes greater than 50,000 MW to be concentrated in a volume as small as five liters. This method, when employed at Station 11A in October 1974, did not yield isolation of authentic *Salmonella*. However, four cultures presumptively identified as *Salmonella* were isolated. The possibility does exist that *Salmonella* spp. recovered from environmental samples, in which they have resided for a very long period of time, may be physiologically and/or serologically distinct from the *Salmonella* spp. in the gut of animals and man. If this turns out to be the case, present methods of enumeration of *Salmonella* are ill-designed for identifying these pathogens in estuarine samples. A nonselective enrichment procedure proved successful in isolating *Salmonella*. (See Appendix B.)

Clostridium botulinum - The incidence of botulism poisoning in man in the United States is extremely low (Gangarosa et al., 1971). Yet, there exists the capacity for serious epidemics through improper preservation of foods or the use of newer, unproven food preservation methods. Furthermore, botulism has been the cause of large-scale wildfowl mortalities. The association of botulism with alewife mortalities in the Great Lakes has been suggested (Graikoski et al., 1969). The majority of human mortalities in the United States has been associated with types A, B, and E toxins. Types A and B are restricted, generally speaking, to the western and eastern sections of this country, respectively, and Type E is associated primarily with aquatic habitats (Gangarosa et al., 1971). The Upper Bay Survey provided an excellent opportunity to search for this organism and to assess a possible role in the mortalities of various species of animals in the Chesapeake Bay (See Table 3-5). The incidence of the organism in samples of sediment was found to be sporadic. Of the 41 samples collected, four were found to be positive for *Clostridium botulinum*. Two of six samples from Station 1A were found to be positive, and Type B and E toxin were detected. The simultaneous detection of *Cl. botulinum* in sediment and in the victim of a fishkill in June 1974 may prove to be significant. A number of unusual circumstances characterized the upper bay in June. The water was unusually clear; the densities of plankton were very low and an oxygen depletion was measured below the halocline.

In view of the fact that approximately 10 percent of all samples examined were positive for lethal toxin production, the assessment of *Clostridia* in the upper Chesapeake Bay should be considered a significant public health problem; further study is clearly warranted. Fully one-third of the samples collected at Station 1A were found to be positive, suggesting the need for a careful study of the source of such contamination and its implications to the shellfish industry.

Vibrio parahaemolyticus - *Vibrio parahaemolyticus* is a recognized agent of food borne illness (Sakazaki, Personal Communication) and has been shown to be pathogenic for oyster larvae (Tubiash et al., 1970). The organism is detected in the waters of mid-Chesapeake Bay when the water temperature is above 15°C, and it has been shown to be associated with the surfaces of copepods (Kaneko and Colwell, 1973). *V. parahaemolyticus* was enumerated in this study so the distribution of an estuarine pathogen could be compared with the distribution of organisms associated with domestic sewage.

Three different isolation procedures for *V. parahaemolyticus* were employed and, in each case, numbers of *V. parahaemolyticus*-like organisms (VPLO) were found to increase from Station 1A to Station 11A (See Table 3-3). This is consistent with the known habitat and salt requirements of this organism. That is, *V. parahaemolyticus* is an estuarine organism with a requirement for salt. Few confirmed *V. parahaemolyticus* strains were isolated during the winter and spring, a finding in keeping with the temperature limits shown for this organism in an earlier study undertaken in the University of Maryland laboratory (Kaneko and Colwell, 1973). Direct plating on TCBS* agar was found to be the most selective. However, reduced recoveries may result without prior incubation in less selective media (T. Kaneko, PH. D. Thesis, Georgetown University, 1973).

3.3.8 Bacteriology of Oysters

Total viable counts of oyster tissue did not exceed State of Maryland limits for shell stock. (Maximum permitted is 100,000 TVC per 100 g of oyster meat.) At not time did the fecal coliform MPN of oysters exceed the State recognized standard of 130 fecal coliforms per 100 g (Personal communication, M. J. Garreis, Maryland Environmental Health Administration). Fecal streptococci were shown to be three to ten times higher in oyster tissue than fecal coliforms. However, there are no recognized max-

*TCBS is thiosulfate citrate bile salts sucrose

imum limits for fecal streptococci in shellfish. Fecal streptocci levels in oyster tissue were comparable to those found in sediment samples collected at Station 11A during the summer (See Figure 3-8 and Table 3-9). No *Salmonella* spp. or *Cl. botulinum* were found to be present in oysters from September to December 1974. However, this does not indicate an inability to retain these pathogens, since pathogens were not found in the overlying water during the same period. One presemptive VPLO was recovered from oysters collected during September. This isolation was coincidental with five presumptive VPLO isolations from water taken at Station 11A during the same time. The positive VPLO isolation occurred during a time of high coliform and fecal streptococci density in water from Station 11A. The probability exists that pathogen uptake and retention by shellfish may be dependent on a threshold concentration of such organisms in their immediate environment. In general, all bacterial counts appeared to decline as winter approached (i.e., as the water temperature dropped).

3.3.9 PCB-Metabolizing Bacteria

Significant proportions of the TVC of water and sediment were recovered from media containing PCB 1254 as the primary source of carbon for growth (Table 3-10). Approximately one to ten percent of the total viable bacterial population may be involved in the potential metabolism of PCB 1254 in the water of the upper Chesapeake Bay. A somewhat lower proportion (0.1% $\geqslant$ 10%) of the TVC of sediment samples was capable of growth on PCB 1254. However, in absolute numbers, this represents a greater concentration of bacteria, roughly 10 to 100 times more numerous than in the water column. The results obtained for upper Chesapeake Bay water and sediment samples corresponded well to similar estimates of PCB-metabolizing bacteria found in the marine environment (Atlantic Ocean). In general, the total number of PCB-metabolizing bacteria was lower in the upper bay than in Miami harbor (Station 1E), but higher than in the open ocean (Stations 3E to 9E).

Six bacterial genera were identified among isolates from samples collected at the upper Chesapeake Bay stations (Table 3-11). Of 26 isolates, 11 (42%) were identified as Pseudomonads, 6 tentatively as *Aeromonas* spp., 5 as *Bacillus*, and the remaining 3 organisms tentatively as *Streptomyces, Micrococcus,* and *Acinetobacter*. There appeared to be a transition from Gram negative PCB-metabolizing bacteria in the Conowingo (Havre de Grace) area to a Gram positive population of PCB-metabolizing bacteria in samples collected further down the bay. This transition in bacterial genera proceeding down the bay may be an indication of the type and composition of various pollutants entering the Chesapeake Bay. Further clarification of this point may be possible when upper Chesapeake Bay nutrient data and chlorinated hydrocarbon distributions at upper bay stations are compared with the results of the microbiological analyses.

Evidence of PCB 1254 effect on bacterial activity was obtained using a [U—^{14}C] glucose decomposition assay, whereby differences were measured in the metabolic activity between populations of bacteria in marine habitats (Figure 3-12). A multivariate analysis of variance for non-replicated samples was performed on the ^{14}C-glucose decomposition assay data obtained during R/V *EASTWARD* Cruise, E-1613-74. Results of the statistical analysis indicated no significant stimulatory or inhibitory effect in the overall dissimilation of [U—^{14}C] glucose. There was a significant variability between sampling stations, attributable to the populations and proportions of PCB-metabolizing bacteria in the individual samples (See Table 3-10).

3.3.10 Effect of PCB-1254 on Bacterial Activity

Initial evidence indicating microbial utilization of the biodegradable PCB 1254 was the isolation of bacteria from enrichment broths containing PCB 1254 as the sole carbon source. The question remained as to whether these bacteria were actually metabolically active in breaking down PCB 1254. *Pseudomonas* Strain 1008, isolated from enrichment flasks with an inoculum from a Conowingo sample, was selected for further study of the effect of PCB 1254 on bacterial activity. Preliminary results showed that Culture 1008 is capable of growth in flasks containing 1,000 mg/1 of PCB 1254 on

TABLE 3-9. COMPARATIVE BACTERIOLOGY OF OYSTERS* AND BOTTOM WATER COLLECTED AT TOLLY BAR, STATION 11A

Bacteriological Parameter	September		October		November		December	
	Oysters	Water	Oysters	Water	Oysters	Water	Oysters	Water
Total viable count**	6.05×10^2	4.5×10^3	7.5×10^3	6.9×10^3	--	1.8×10^4	9.4×10^0	1.2×10^4
Indicator organisms***								
Total coliforms	79.0	22.0	46.0	7.8	< 4.6	4.5	4.0	33.0
Fecal coliforms	< 1.8	< 1.8	4.0	2.0	< 4.6	< 1.8	4.0	4.5
Fecal streptococci	15.0	92.0	46.0	1.8	12.4	0.6	14.6	3.0
Pathogens								
Clostridia†	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Salmonellae††	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
VPLO†††	1.0	5.0	0.0	2.0	0.0	1.0	0.0	1.0

* Ten oysters per sample, 100 to 200 g oyster tissue.

** TVC/g oyster tissue or ml^{-1} bottom water.

*** MPN/100 g oyster tissue or 100 ml^{-1} bottom water.

† *Clostridia botulinum* toxin, mouse bioassay.

†† Selenite cystein enrichment, MacConkey-Selenite isolation medium.

††† Direct plating on TCBS medium.

TABLE 3-10. CONCENTRATION OF PCB–METABOLIZING BACTERIA IN SELECTED ESTUARY AND MARINE SAMPLES

Date	Station	PCB–Metabolizing Bacteria per gram or Milliliter			
		Water	% of TVC	Sediment	% of TVC
9/74	1A	--	--	$> 10^2$	0.01
	5A	--	--	$> 10^2$	1.0
	10B	--	--	$> 10^2$	0.1
	11A	--	--	$> 10^2$	1.0
	Conowingo	$> 10^2$	10.0	--	--
10/74	1A	--	--	--	--
	5A	3.5×10^1	6.3	4.5×10^2	3.6
	10B	4.0×10^1	8.2	4.5×10^2	0.4
	11A	6.5×10^1	0.9	1.3×10^3	0.3
11/74*	1E	1.3×10^2	22.8	9.5×10^3	4.5
	3E**	1.0×10^2	5.0	3.5×10^2	2.9
	5E**	1.0×10^2	6.3	4.5×10^1	0.9
	7E**	1.0×10^2	2.6	1.2×10^3	46.0
	8E	1.3×10^1	> 100.0	1.2×10^2	46.0
	9E	6.2×10^1	> 100.0	5.4×10^2	13.5
	10E	7.0×10^1	0.09	3.9×10^3	3.3

*Samples obtained from Miami Harbor to Cape Hatteras, N. Carolina along the outer continental shelf.

**Surface water counts elevated due to carryover contamination from 1E in continuous flow concentrator.

TABLE 3-11. ISOLATION OF PCB–METABOLIZING BACTERIA FROM UPPER BAY STATIONS

Station	Tentative Genera	Sample Type	Isolation Method
Conowingo	*Aeromonas*	Water	Enrichment
	Aeromonas	Water	Enrichment
	Pseudomonas	Water	Enrichment
	Pseudomonas	Water	Enrichment
	Pseudomonas	Water	Enrichment
	Micrococcus	Water	Enrichment
	Pseudomonas	Water	Enrichment
1A	*Aeromonas*	Sediment	Enrichment
	Aeromonas	Sediment	Enrichment
	Aeromonas	Sediment	Enrichment
	Pseudomonas	Sediment	Enrichment
	Pseudomonas	Sediment	Enrichment
	Pseudomonas	Sediment	Enrichment
	Acinetobacter	Sediment	Enrichment
5A	*Vibrio/Aeromonas*	Sediment	Direct plating
	Pseudomonas	Sediment	Direct plating
10B	*Bacillus*	Sediment	Enrichment
	Bacillus	Sediment	Enrichment
	Bacillus	Sediment	Enrichment
	Pseudomonas	Sediment	Enrichment
	Pseudomonas	Sediment	Enrichment
11A	*Bacillus*	Water	Direct plating
	Streptomyces	Sediment	Direct plating
	Pseudomonas	Oyster	Direct plating
	Bacillus	Water	Direct plating

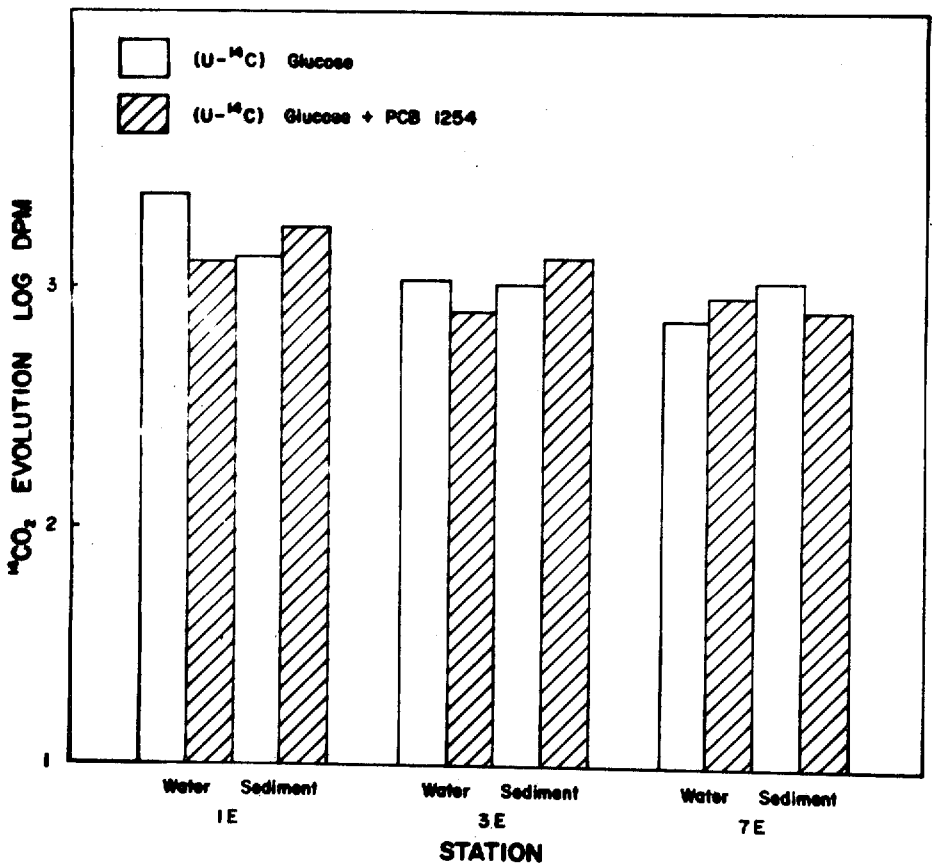

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Figure 3-12. Effect of PCB 1254 on the Dissimilation of [U-^{14}C] Glucose by a Heterogenous Marine Population

diatomaceous earth. Additional data were obtained showing that other cultures were capable of emulsifying droplets of PCB 1254 adherent to the bottom of enrichment flasks. Such emulsification at times reached 100 percent, resulting in the formation of an oil film on the surface of the enrichment broth.

The rate of oxygen consumption of Strain 1008 was found to be enhanced by concentrations of PCB 1254 less than 1,000 mg/l (Figure 3-13). There was no significant change in oxygen uptake for Culture 1008 grown on Celite or on Celite coated with PCB 1254 at 1,000 and 2,000 mg/l. Progressively lower concentrations of PCB 1254 significantly increased oxygen consumption. Since oxygen uptake is a function CO_2 evolution in differential respirometry, increased oxygen uptake must result from increased CO_2 evolution. Increased CO_2 evolution, in this case, may result from stimulated decomposition of organic matter associated with the diatomaceous earth or from active decomposition of PCB 1254.

Further evidence for the stimulation of oxygen uptake by PCB 1254 or for degradation of PCB 1254 was shown in Figure 3-12. A greatly increased rate of oxygen consumption was observed when glucose (1,000 mg/1) was supplemented with PCB 1254 (1,000 mg/). This increased oxygen uptake was significantly higher than that obtained for the Celite control or the glucose-coated Celite (Figure 3-14). The lag in oxygen consumption between zero and 12 hours in the glucose-PCB curve was similar to the PCB-induced lag in growth rate observed by Bourquin and Cassidy (1974).

Humic acid - Humic acids are a group of ill-defined, stable, natural polymeric substances thought to be derived from the biodecomposition and biomodification of organic material of primarily plant origin. Because of their polyfunctional, polar nature, they are capable of complexing a wide variety of inorganic and organic substances, including heavy metals and pesticides (Stevenson, 1972). For this reason, it was felt that humic acid (HA) might be involved in the transport of bacteria and nutrients, as well as chlorinated hydrocarbons.

Humic acids were extracted and measured from samples of water and sediment collected during the December and January cruises. The results of this initial survey are presented in Table 3-12. Humic acid in both water and sediment increased from Station 11A to Station 1A at the head of the bay, suggesting that the humic acid is primarily of terrestrial origin. Humic acid in the water samples was predominantly particulate in nature. These preliminary results indicate that the potential complexing capacity of the water and sediment is greater at the head of the bay. If this is so, the flux of materials associated with humic acid should decrease down the bay.

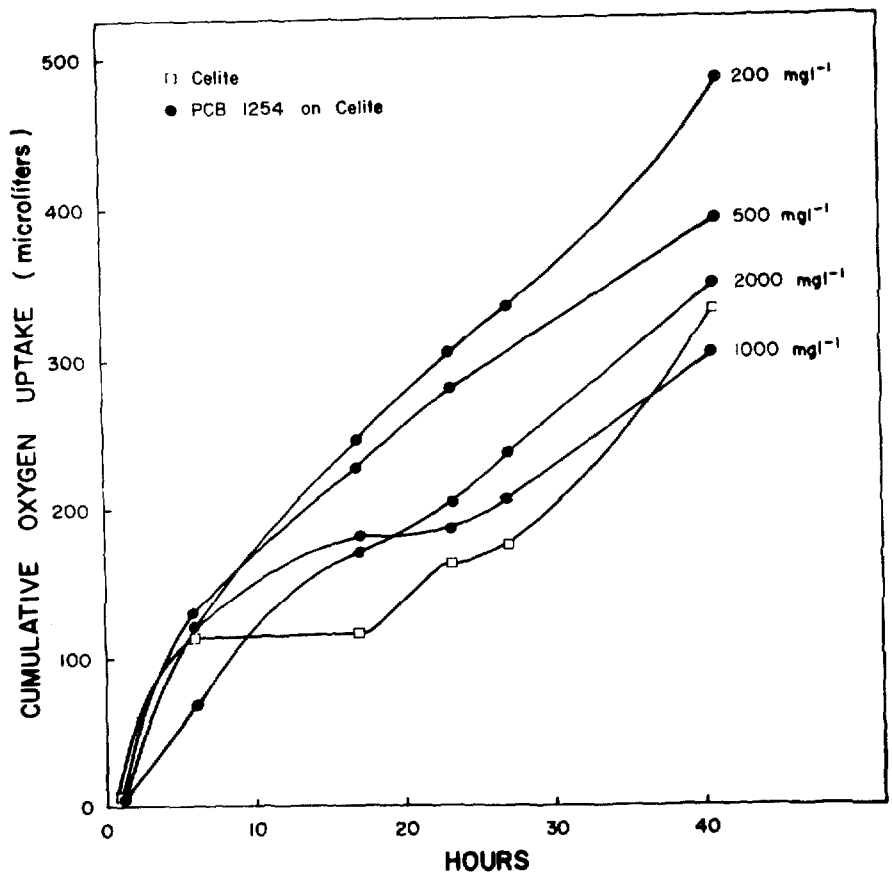

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Figure 3-13. Oxidation of PCB 1254 by an Estuarine Pseudomonas spp

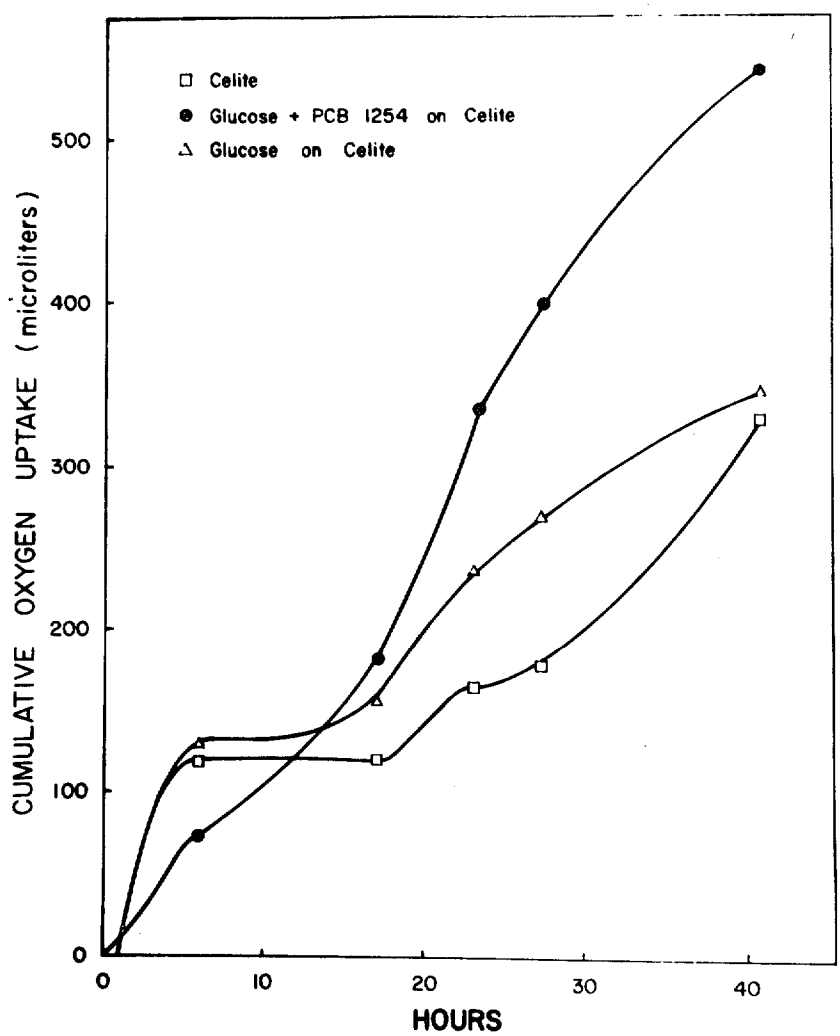

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Figure 3-14. Effect of PCB 1254 on Glucose Oxidation by an Estuarine Pseudomonas spp

TABLE 3-12. CONCENTRATION OF HUMIC ACID IN UPPER CHESAPEAKE BAY

Date	Station	Humic Acid Concentration: Sediment Micrograms/g Dry Weight	Water (Micrograms/Liter) Particulate	Water (Micrograms/Liter) Dissolved
12/73	11A	108	150	70
	10B	639	229	143
	5A	1175	520	120
	1A	2880*	800	225
1/74	11A	450	—	—
	10B	757	180	—
	5A	642	240	—
	1A	15**	600	—

*Silty sediment.

**Sand.

3.4 Conclusions

In view of the diversity and volume of data collected on the microbiological parameters of pollution in the upper Chesapeake Bay, a summary of the conclusions derived from this study may assist the reader in interpreting the results:

(1) The high bacterial TVC's observed to occur during winter and early spring would indicate that an influx of organisms and/or nutrients occurs in the upper bay at that time.
(2) Station 1A at Havre de Grace appears to vary independently from the other stations, most likely a reflection of the impact of the Susquehanna River.
(3) Station 1A, the data for which represented the highest influx of all of the bacterial indicator groups studied, receives point source pollution compounded by the contribution of organisms from the Susquehanna River.
(4) The Susquehanna River below Conowingo Dam contributes large numbers of allochthonous bacteria from non-human sources to the upper Chesapeake Bay.
(5) Judging from high MPN levels and elevated FC/FS ratio, contamination of a domestic source enters the upper bay, most likely in the area of Station 1A.
(6) Suspended sediment serves as a vehicle of transport for a large proportion of the viable aerobic, heterotrophic bacteria in the water column. In addition, many indicator organisms are associated with suspended sediment, perhaps originating at the source of pollution.
(7) The relative abundance of indicator organisms, which increases in summer months, reflects seasonal effects of temperature and nutrients, and possibly, increasing activity on the bay during this time.
(8) Significant populations of indicator organisms are associated with bottom sediments. Resuspension or movement of sediment may result in the exchange of large numbers of total coliforms and fecal Streptococci with the surrounding water and suspended sediment.
(9) A potential public health problem may exist in Chesapeake Bay since the presence of *Clostridium botulinum* in upper bay sediments has been shown in this study.
(10) Shellfish at Tolly Bar are acceptable for public consumption by the criteria currently employed. There appears to be a less dramatic uptake of indicator organisms from Station 11A sediments or water than was expected.
(11) *Salmonella* and *Vibrio parahaemolyticus*-like organisms were isolated employing specially designated isolation methods. Difficulty encountered in enumeration may mask the significance of these organisms in upper Chesapeake Bay water and sediment.
(12) Bacteria capable of metabolizing PCB's are distributed throughout the Chesapeake Bay. The apparent transition of bacterial genera descending down the bay may be indicative of the nature of pollution, i.e., industrial vs. domestic pollution.
(13) The effect of PCB 1254 can be stimulatory for some strains of bacteria; however, only a small portion of the total viable bacterial population was capable of growth in the presence of PCB 1254.

3.5 Acknowledgements

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APPENDIX 3A

EFFECT OF POLYCHLORINATED BIPHENYL ON THE ACCUMULATION AND RETENTION OF ENTERIC BACTERIA BY THE OYSTER, *CRASSOSTREA VIRGINICA*

3A1. INTRODUCTION

Polychlorinated biphenyls (PCB) are a recognized contaminant of estuarine and coastal marine water throughout the world (Duke et al. 1970; Risenbrough et al., 1968; Veith and Lee, 1970). These industrial chlorinated hydrocarbons (CHC) have been shown to be acutely and chronically toxic for many estuarine and marine fishes and invertebrates (Duke et al., 1970; Hansen et al. 1974; Hansen et al. 1971). However, little information is available concerning secondary levels of impact of PCB contamination on estuarine and marine animals. A secondary level of impact includes PCB-induced stress, altering the normal physiology of the animal, and rendering it vulnerable to invading parasites or pathogens.

The primary objective of this investigation was to determine whether PCB-induced stress on the oyster, *Crassostrea virginica,* caused it to accumulate enteric bacteria. A study was undertaken to test the hypothesis that PCB concentrations commonly encountered by estuarine invertebrates may result in reduced bacteriological quality of a commercially important shellfish. It has been shown by other investigators that the oyster can effectively filter pathogenic bacteria and viruses from overlying waters and accumulate significant quantities of these microorganisms in tissue and on gill surfaces (Fugate et al., 1975; Janssen, 1974). Retention of enteric or pathogenic bacteria in stressed oysters could lead to serious economic, as well as public health situations, if commercial oyster beds should be closed as a result of high coliform counts arising from PCB or other stress, and not from sewage contamination.

3A2. MATERIALS AND METHODS

3A2.1 Culture Conditions

Laboratory investigations were conducted using two bacterial strains, one an indicator of contamination by domestic sewage and the other a known pathogen. They were *Escherichia coli* Type I, isolated from the upper Chesapeake Bay and a laboratory stock culture of *Salmonella typhimurium* respectively.

Bacterial cultures were harvested by centrifugation at 16,300 x g after growth for 48 hours in nutrient broth. Pelleted cells were resuspended in sterile salts broth. The resuspended cells were divided into equal portions and used for inoculation of aquarium water in tanks containing oysters.

Analysis of oyster tissue, following dosing with the bacteria, was according to American Public Health Association procedures (APHA, 1970). Oyster shell surfaces were disinfected with 2.5 percent hypochlorite in an ice bath. Oysters were shucked; the tissue was excised, rinsed with phosphate buffered saline, weighed, and homogenized with 100 ml 0.5 percent peptone.

Total viable bacterial counts (TVC) of both the oyster homogenate and the aquarium water were performed using UBYE (Sayler et al., 1975) agar and appropriate dilutions of the samples. Quantitative *E. coli* determinations were made employing MacConkey agar. Since there was an absence of lactose-fermenting organisms prior to *E. coli* dosing, all lactose-positive cultures growing on MacConkey agar were recorded as *E. coli.* At high concentrations of *E. coli,* water or tissue dilutions were plated directly on MacConkey agar. As the number of *E. coli* dropped, membrane filters (Millipore Corp., New Bedford, Mass.) were used to concentrate the bacteria. The filters were placed on the surface of MacConkey agar plates and incubated at 37°C for 24 to 48 hours.

A similar procedure was used for estimation of *Salmonella typhimurium,* except that bismuth sulfate agar (BSA) was used for enumeration. Green colonies on BSA, after 24-hour incubation at 41°C, were recorded as *Salmonella.* Additional confirmatory tests were made on Kliegler iron agar, as warranted, to determine if biochemical alteration of the *S. typhimurium* occurred as a result of exposure to PCB.

PCB stress was simulated using Aroclor 1254® (Monsanto Industrial Chemicals, St. Louis, Mo.), coated on diatomaceous earth (Celite, J. T. Baker Chemical Co., Phillipsburg, N.J.). PCB dosing was maintained at 10 mg per liter (100 mg per liter Celite) for all experimental work.

3A2.2 Oyster Maintenance

Oysters (*Crassostrea virginica*) used in this study were dredged from Tolly Bar in the southernmost part of upper Chesapeake Bay near Annapolis, Maryland. This area of the Chesapeake Bay—including water, sediment, and oysters harvested in this area— has been found free of enteric pathogens and is judged fit for shellfish harvesting (Sayler et al., 1975). Each animal collected received a preliminary cleaning aboard ship to remove mussels and associated animals from the shell. All oysters were transported to the laboratory and stored at 6°C within six hours of collection. Experimental work was initiated within 72 hours of collection.

Oysters were maintained in 60-gallon, custom-designed, recirculating refrigerated aqaria (Sea Lake Systems, Inc., Euclid, Ohio). Operating temperature was maintained at 15°C. Each aquarium was sterilized by autoclaving in an AMSCO®steam autoclave (American Sterilizer Corp, Erie, Pa.). Two hundred liters of steam-distilled water were filtered through 0.45 μm, 90 mm Millipore membrane filter (Millipore Filter Corp., Bedford, Mass.) and added aseptically by gravity flow to each aquarium. Artificial sea salt (Sea Lake Systems) was autoclaved in the dry state and was added to each aquarium, to a final salinity of 12 $^0/_{00}$, approximately equal to the *in situ* salinity at Tolly Bar. Each aquarium was fitted with glass covers to reduce or eliminate potential contamination. Refrigerant coils and air lines were disinfected with 2.5 percent hypochlorite prior to each experiment.

One hundred randomly-sized oysters were selected from the total set of oysters collected. Shell surfaces were thoroughly cleaned with a wire brush, and each animal was surface-disinfected by placing it in an ice bath, followed by an iced 2.5 percent hypochlorite bath for three to five minutes. Icing was employed to insure that each animal remained tightly closed; hence, disinfectant was prevented from reaching the tissue of the animal. In each of two aquaria 50 cleaned and disinfected oysters were placed. The oysters were allowed to remain in the aquaria for 48 hours in order to become equilibrated to the system.

Following the equilibration period, one group of oysters received a dose of 10 mg per liter PCB 1254 coated on 100 mg per liter celite. The duplicate aquarium received a placebo of 100 mg per liter celite and, therefore, served as the control for the experiment. Both sets of oysters were held under identical conditions except for stress induced by addition of PCB. Ninety-six hours after PCB dosing, five oysters were aseptically removed from each tank, disinfected, and assayed for bacterial quality according to APHA procedures (APHA, 1970). After removal of the five control oysters, both tanks received a dose of a washed bacterial suspension. Then five oysters were removed from each tank, disinfected, and assayed for accumulated bacteria. Sampling of oysters and water from both aquaria proceeded at established time intervals for 12 days. Next, the remaining oysters from both tanks were removed, surface-disinfected, and placed in separate sterile aquaria. Excretion of the accumulated bacteria was followed in water of the aquaria to which the oysters had been transferred, and purging of bacteria from the animals was determined by periodic sampling of the oysters.

3A3. RESULTS AND DISCUSSION

Two sets of experiments were completed, each involving accumulation, retention, and survival of enteric bacteria. The first set was designed to examine the accumulation, retention, survival, and release of *E. coli* in the oyster tissue and the removal and survival of *E. coli* in aquarium water under conditions of no stress and under conditions of PCB stress.

An outline of the experimental procedure is given in Table 3A-1. Due to a faulty aquarium, excretion of *Salmonella* could not be fully assessed in the second group of experiments. However, the experimental procedure allowed for the study of accumulation and retention of *Salmonella* and a partial study of the release of *Salmonella* by *Crassostrea virginica.*

3A3.1 PCB Stress and the Accumulation of E. coli

The effect of Aroclor®1254 on the survival of *E.coli* in aquarium water is depicted in Figure 3A-1. After *E. coli* addition and four days post-PCB dosing, an *E. coli* concentration of 10^6 cells per liter was reached in both the PCB dosing aquarium and control aquarium. This concentration was maintained for 24 hours in the control tank; however, there was approximately 99 percent reduction in *E. coli* concentration in the PCB-dosed aquarium water.

Within 48 hours, 90 percent of the *E. coli* added to the control aquarium was no longer detectable. The bacteria rapidly declined in the water column in both aquaria thereafter; although, the decline was slightly less pronounced in the control aquarium. Six days following addition of *E. coli* to the PCB-stressed oyster aquarium, *E. coli* concentrations dropped to undetectable levels (less than 1 per ml). *E. coli* were detectable in the control aquarium for an additional four days, indicating a slightly longer survival in the non-PCB-stressed environment.

Comparison of the survival of *E. coli* with fluctuations in total viable counts (TVC), as shown in Figure 3A-1, indicated trends similar to that demonstrated by *E. coli*, with the exception that there was no immediate, marked loss of TVC from the water column. Twelve days after dosing, oysters were removed from the aquaria (Figure 3A-1). Absence of the oysters apparently had only a negligible effect on *E. coli.* However, the TVC increased after the oysters were removed from the aquaria, suggesting that there was growth of heterotrophic bacteria introduced into the aquaria with the oysters.

It is impossible to eliminate all bacteria from the oysters without killing the animals. Therefore, a background TVC, as indicated in counts at initiation of the experiments, must be accepted for all experimental work of this kind.

Oyster tissues assayed for *E. coli* at the time the oysters were removed to fresh aquaria (Day 12) were found to have accumulated large numbers of *E. coli* (Figure 3A-2). There was no significant difference between accumulation of *E. coli* after exposure for 12 days by the stressed and non-stressed oysters. Both groups of oysters accumulated about ten times more *E. coli* than the concentration of *E. coli*

TABLE 3A-1. EXPERIMENTAL OUTLINE FOR ASSAY OF ENTERIC BACTERIA ACCUMULATED BY THE OYSTER, CRASSOSTREA VIRGINICA, FOLLOWING ACUTE PCB STRESS

Days	AQUARIA	
	Stressed	No Stress
-2	50 random oysters (1) 48 hr equilibration	50 random oysters (3) 48 hr equilibration
0	PCB stress	No stress
4	Bacterial does--under stress	Bacterial dose
4-11	Survival and accumulation --under stress	Survival and accumulation
12	Transfer to fresh aquarium --post-stress (2)	Transfer to fresh aquarium (4)
12-19	Survival and release --post-stress	Survival and release

NOTE: The numbers in parentheses above identify the aquaria in which the oysters were placed.

TABLE 3A-2. RELEASE* OF TOTAL VIABLE BACTERIA (TVC) FROM OYSTERS FOLLOWING PCB–STRESS AND E. COLI DOSING

Day	Stressed			No Stress		
	Water**	Tissue††	Percent††† Released	Water**	Tissue†	Percent††† Released
12	2.5×10^2	2.8×10^5	0.007	3.5×10^2	2.6×10^5	0.01
13	5.3×10^3	1.2×10^4	1.9	5.6×10^3	3.6×10^5	1.5
14	4.8×10^3	2.2×10^5	2.0	2.3×10^5	1.2×10^5	190.0
17	1.5×10^6	3.4×10^6	3.8	2.2×10^6	5×10^4	407.0

* Release from oyster to water, assuming no growth in water.

** TVC per ml

† TVC per 100 g of tissue

†† Cumulative percent released, Σ Water/ Σ Tissue x 100

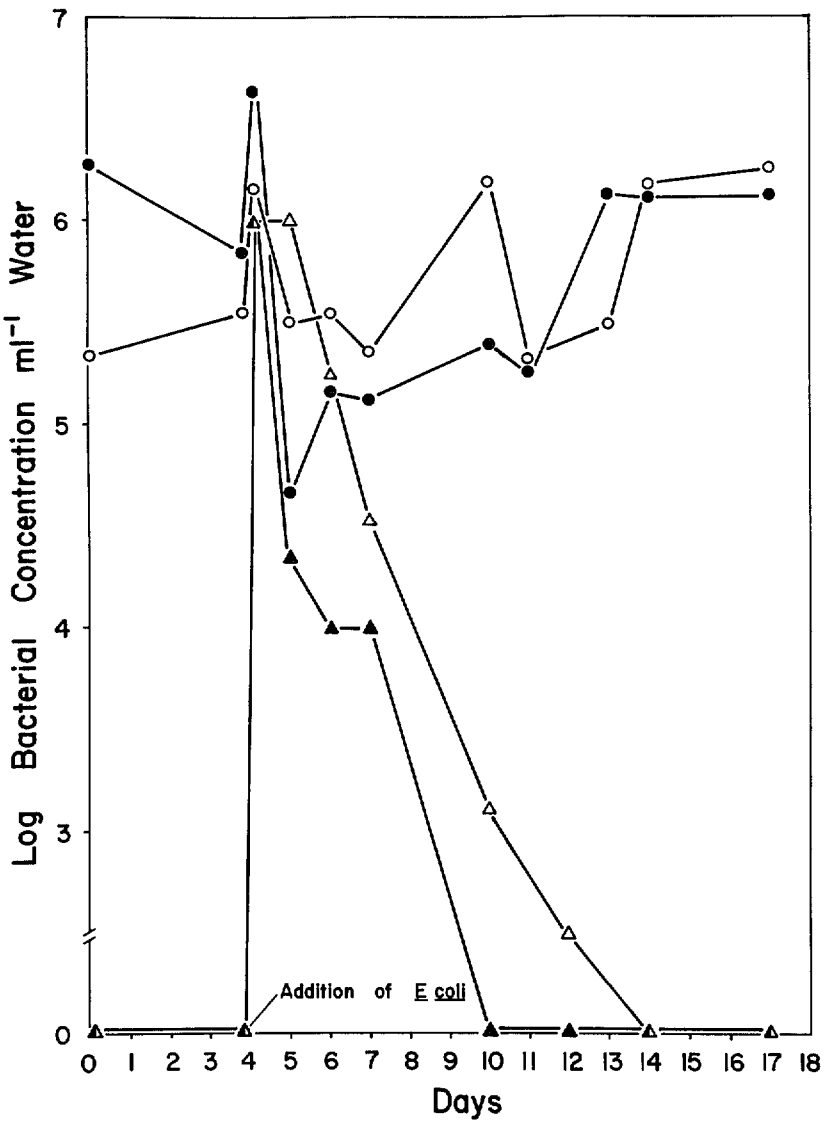

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Figure 3A-1. Survival of Total Viable Bacteria (TVC) and E. coli in Aquarium Water Under PCB-Stress and Unstressed Conditions. (▲ E. coli – PCB Stress, △ E. coli – No Stress, ● TVC – PCB Stress, ○ TVC – No Stress; Oysters Were Removed From the Aquaria at Day 12.)

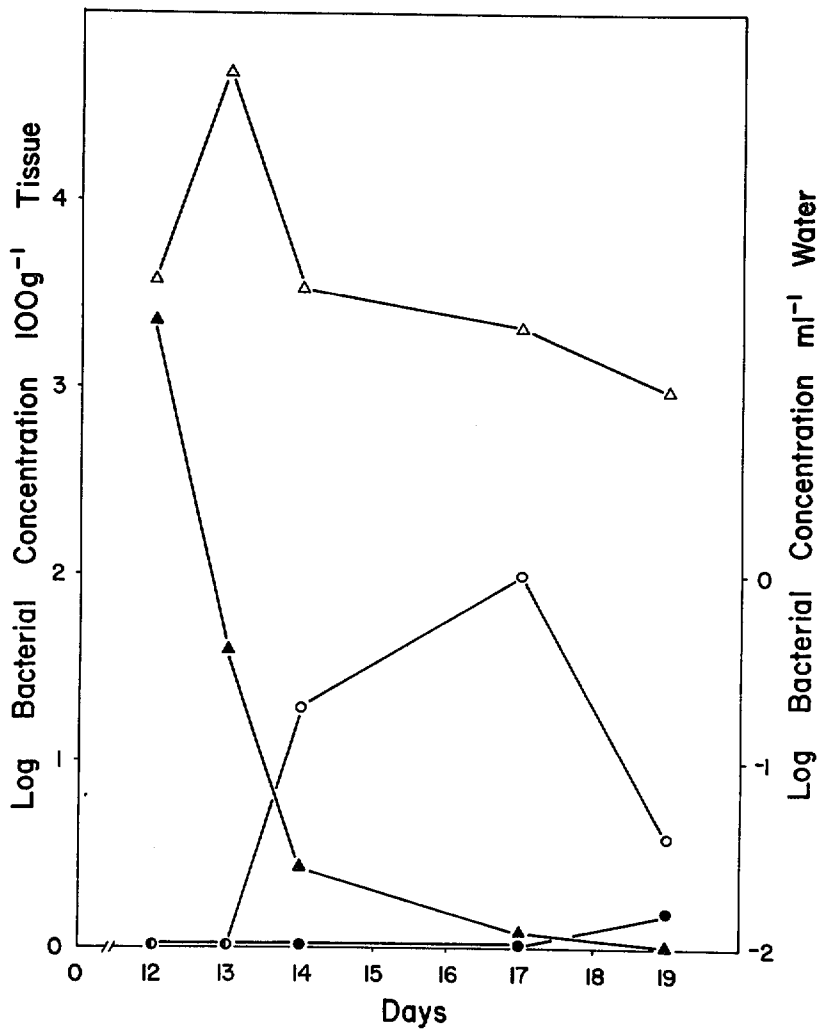

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Figure 3A-2. Accumulation and Excretion of E. coli by Oysters Following PCB Stressed and Unstressed Conditions. (▲ E. coli Tissue Accumulation with PCB-Stress; △ E. coli Tissue Accumulation with No Stress; ● E. coli Excretion with PCB-Stress; ○ E. coli Excretion with No Stress).

added to the aquarium water (Figures 3A-1 and 3A-2). There was significantly greater survival of *E. coli* in the non-stressed oysters, from Day 12 through Day 19, compared with stressed oysters, which eliminated all accumulated *E. coli* by Day 19. The peak in *E. coli* accumulation, occurring at Day 14 in the non-stressed oysters, was most likely due to experimental error or may have been growth of *E. coli* in the oyster tissue, a rather doubtful but not impossible situation.

Elimination of *E. coli*, as can be seen in Figure 3A-2 was interesting, in that those *E. coli* lost from stressed oysters were not recovered in the aquarium water. The resulting conclusion is that these cells were no longer viable. However, in unstressed oysters, *E. coli* was recovered in the water at Day 14, corresponding to the marked loss of *E. coli* from unstressed oyster tissue. Although *E. coli* recovered from aquarium water was an insignifcant amount of the total accumulation of *E. coli* by the oysters (<1.0%), it was significantly more than was recovered from oysters dosed with PCB 1254.

The depressive effect of PCB 1254 on the elimination (or depuration) by the oyster of total viable bacteria was clearly evident (Table 3A-2). Elimination of the viable heterotrophic bacteria by PCB-stressed oysters amounted to a maximum of 3.8 percent of the total bacteria accumulated, compared with 407 percent for the control oysters; although, initial accumulation of TVC was approximately the same.

These data support two theories: (1) *E. coli* is sensitive to PCB 1254 and (2) the ability of the oyster to accumulate bacteria is not inhibited by PCB, but depuration is diminished.

3A3.2 PCB Stress and the Accumulation of Salmonella Typhimurium

Accumulation of *S. typhimurium* by stressed and unstressed oysters revealed patterns similar to those of *E. coli*, with some exceptions. It was immediately obvious that the quantitative rate of recovery of *Salmonella*, using bismuth sulfite agar was much less than that of *E. coli*. This was evident from the discrepancy observed between TVC and the numbers of recovered *Salmonella* following addition of more than 10^6 cells per ml to the water of each aquarium (Figure 3A-3). However, results for groups of oysters receiving the same treatement would not be affected by the problem of quantitation.

As was noted for *E coli* (Figure 3A-1), the number of *Salmonella* in the aquarium water decreased rapidly, starting at the time of addition of the bacteria four days after PCB dosing. There was a slight difference between decline in *Salmonella* levels between eight and twelve days while the aquarium water without PCB showed what could be interpreted as growth of the *Salmonella* paralleled with a rise in total viable bacteria. Both increases ceased at the thirteenth day, with a precipitous drop in the number of *S. typhimurium* in the control aquaria. In general, the decline in the number of *Salmonella* was much less gradual than that noted for *E. coli* although the length of time during which a detectable number of viable cells could be recovered was approximately the same, i.e., ten days. The total viable counts followed closely the trends observed for *Salmonella,* with higher TVC concentrations detected in the unstressed environment.

It was not possible to follow depuration of *S. typhimurium* because of a defect in one of the aquaria preventing removal of the oysters to a fresh, sterile environment for purging experiments. It was possible, however, to assay accumulation of *S. typhimurium* in the presence of low levels of residual *Salmonella* in the initial dosing tanks.

At Day 6, oysters in both environments accumulated approximately one-tenth the concentration of cells present in the surrounding water (Figures 3A-3 and 3A-4).Minor loss of *Salmonella* from control oysters between Days 6 and 12 may have been responsible for the observed increase in concentration of *Salmonella* in the water (Figure 3A-3). As the concentration of *Salmonella* in the water declined following Day 12 (Figure 3A-3), a dramatic reduction in the concentration of *Salmonella* in the tissues occurred (Figure 3A-4). The results indicated that depuration of *Salmonella* by the oyster occurred.

Comparisons between accumulation of *Salmonella* by stressed and unstressed oysters are presented in Table 3A-3. There was little difference noted between the groups in absolute accumulation of *Salmonella* or in the relative percentage accumulation of *Salmonella*. One difference noted, however, was the high initial rate of accumulation of bacteria by unstressed oysters at Day 6. It is of interest to note that the only deaths of oysters in all the experiments occurred between Days 6 and 14 among the control oysters dosed with *Salmonella*. *Salmonella typhimurium* was recovered from the gut of one of the dead animals. The evidence is suggestive, but not conclusive of death induced by *Salmonella.*

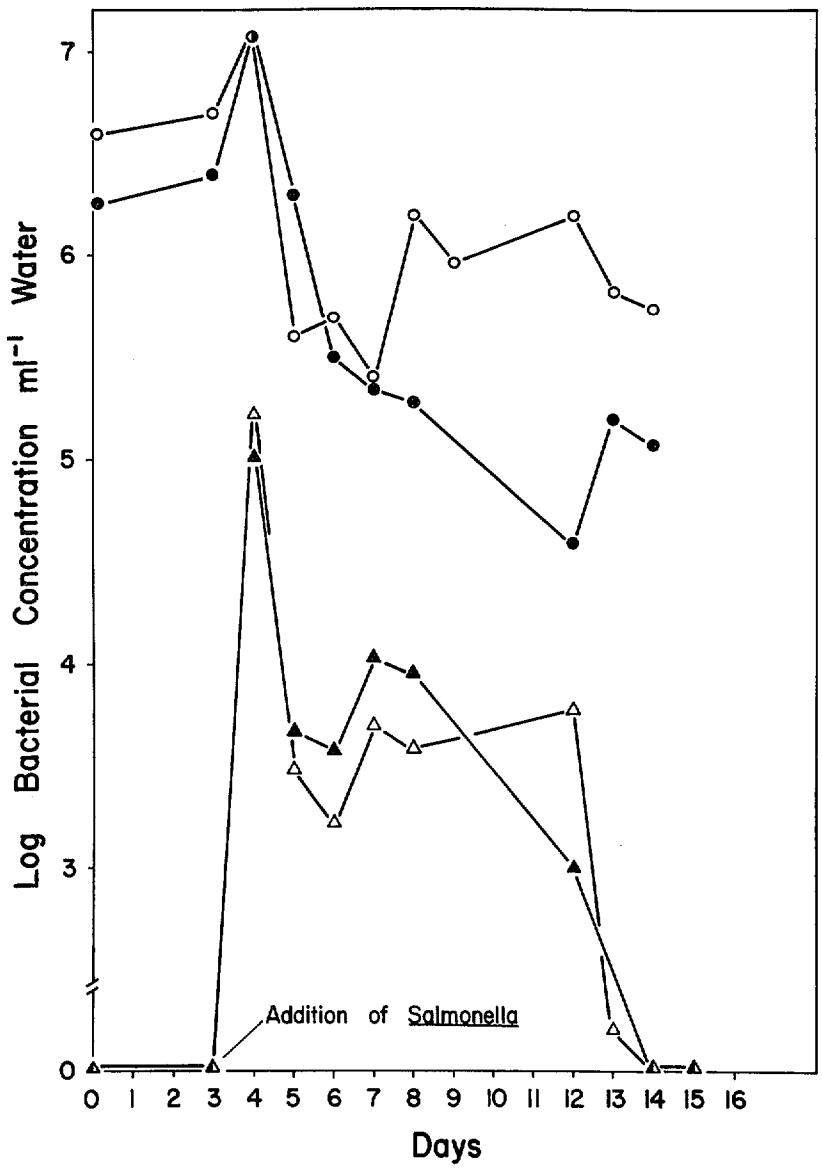

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Figure 3A-3. Survival of Total Viable Bacteria (TVC) and Salmonella Typhimurium in Aquarium Water Under Stressed and Unstressed Conditions. (▲ *Salmonella* with PCB Stress; △ *Salmonella* with No Stress; ● TVC, With PCB Stress; ○ TVC With No Stress; Oysters Removed at Day 12).

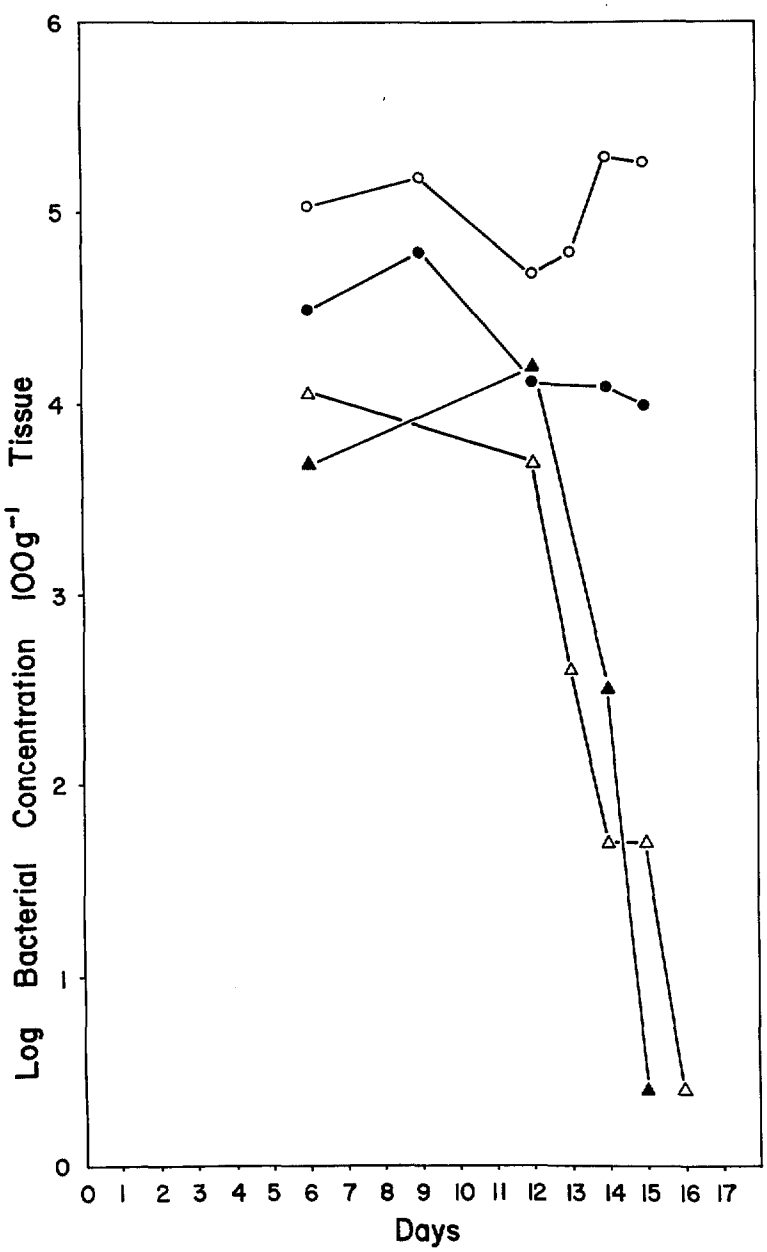

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Figure 3A-4. Accumulation of Total Viable Bacteria (TVC) and Salmonella Typhimurium by the Oyster, Crassostrea Virginica Following PCB Stress (● TVC with PCB Stress; ○ TVC with No Stress; ▲ Salmonella with PCB Stress; △ Salmonella with No Stress).

TABLE 3A-3. ACCUMULATION* OF SALMONELLA TYPHIMURIUM IN OYSTER TISSUE FOLLOWING PCB STRESS

Day	Stressed			No Stress		
	Water**	Tissue†	Accumulated††	Water**	Tissue†	Accumulated††
6	4.4×10^3	4.8×10^3	109.0	1.7×10^3	1.3×10^4	764.7
12	4.7×10^3	1.9×10^4	261.5	7.5×10^3	5.5×10^3	201.1
14	1.0×10^1	3.0×10^2	264.5	2.0×10^2	4.0×10^2	201.1
15	1.0×10^0	2.8×10^1	265.0	3.0×10^1	5.0×10^1	201.0

* Accumulation, assuming no growth of *Salmonella* in tissue

** *Salmonella* per ml aquarium water

† *Salmonella* per 100 g oyster tissue

†† Cumulative percent Accumulated, Σ Tissue/ Σ Water x 100

TABLE 3A-4. ACCUMULATION OF TOTAL VIABLE BACTERIA (TVC) IN OYSTER TISSUE FOLLOWING PCB STRESS AND SALMONELLA DOSING

Day	Stressed			No Stress		
	Water**	Tissue**	Accumulated***	Water*	Tissue**	Accumulated***
6	3.1×10^5	3.1×10^4	10.0	4.6×10^5	1.2×10^5	26.0
9	8.0×10^5	6.3×10^4	8.5	8.0×10^5	1.6×10^5	22.2
12	4.8×10^4	1.4×10^4	9.3	1.6×10^6	5.6×10^4	11.7
13	1.6×10^5	1.6×10^4	9.4	6.8×10^5	6.7×10^4	11.4
14	1.2×10^5	1.3×10^4	9.3	5.6×10^5	2.0×10^5	14.7

* TVC per ml

** TVC per 100 g tissue

*** Cumulative percent accumulated, Σ Tissue/ Σ Water x 100

Accumulation of total heterotrophic bacteria in oyster tissue was greater in unstressed oysters (Figure 3A-4). This observation was made for TVC in the *E. coli* accumulation experiments. In terms of relative percent accumulation of TVC, accumulation by the control oysters ranged from 26 to 11.4 percent of the total number of *Salmonella*, compared with 10.0 to 8.5 percent for PCB-stressed oysters (Table 3A-4).

Janssen (1974) reported oyster retention of 2.8×10^4 *S. typhimurium* per oyster from water containing 2×10^5 cells per ml after 48 hours exposure. Although it is difficult to compare the results reported by Janssen on the basis of per oyster accumulation, it does appear that the results of this investigation are comparable in the case of the unstressed oysters.

Several preliminary conclusions can be drawn from the results of these investigations to date. As expected, the oyster, *Crassostrea virginica*, demonstrated an ability to concentrate enteric bacteria. The effect of PCB stress on oysters apparently is a more complex process than was initially considered in terms of bacterial accumulation and depuration. A stress appears to be imposed on the bacterial population, as well as on the oyster. The end result is that PCB stress artificially produces what superficially could be considered oysters of higher bacteriological quality than was, in fact, the case. Upon harvest, it is possible that the oyster, held under conditions conducive to bacteria growth, would demonstrate marked increases in total count and, correspondingly, coliform count. In fact, this may explain in part the anomalous conditions observed in the Chesapeake Bay when harvested shellfish yield unexpectedly high coliform counts. Effects of temperature and other factors during storage and shipping also must be considered, of course. The latter was not part of the study reported here.

This observation was totally unexpected, as can be judged from the foregoing hypothesis in which it was assumed that PCB stress would result in poorer bacteriological quality. On the other hand, indications are that PCB stress may result in a lessening of the ability of the animal to purge itself of bacteria. These observations require further study before they can be accepted as fact. In the interim, experimental work continues with the soft shell clam, another estuarine invertebrate, as the test animal to determine whether the effects observed for the oyster are specific or are applicable to other estuarine animals.

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APPENDIX 3B

ENUMERATION AND ISOLATION OF *SALMONELLA* IN THE ESTUARINE ENVIRONMENT USING NON SELECTIVE ENRICHMENT

3B1. INTRODUCTION

Results of recently published studies accomplished in our laboratory have shown that large populations of fecal indicator organisms can be found in the water and bottom sediment of Chesapeake Bay and its sub-estuaries (Carney et al., 1975, Sayler et al., 1975). However, demonstration of the presence of enteric pathogens, specifically *Salmonella* spp., was not successful (Carney et al., 1975; Sayler et al., 1975). It was postulated that a low frequency of occurrence of *Salmonelleae,* combined with an inefficient method for enumeration of these organisms in the estuarine environment, were responsible for failure to detect authentic *Salmonella* spp. in areas where large populations of fecal coliforms were present (Sayler et al., 1975b).

An investigation was undertaken to evaluate available methods for enumeration and isolation of *Salmonella* spp., particularly with application to the estuarine environment. Also, an objective of the study was to investigate the relationship between occurrence of enteropathogenic bacteria and their associated indicator organisms.

3B2. MATERIALS AND METHODS

3B.2.1 Sampling

Thirty-four Chesapeake Bay water and sediment samples were collected during the period March 31, 1975 to June 20, 1975. The samples were collected at eleven sampling stations located in an area from Baltimore, Maryland to Norfolk, Virginia (Figure 3B-1).

Water samples were collected using two sampling techniques. Bottom water was sampled at one to two meters above the sediment-water interface with a two-liter sterile Niskin bag sampler (General Oceanics, Miami, Fla.). Surface water samples were pumped on board ship via a submersible pump into 10 percent formalin-rinsed holding tanks. The submersible pump and holding tanks were rinsed with water from the given sampling site prior to the collection of the sample to be analyzed. Surface water was then concentrated from a volume of 100 gallons (about 400 liters) to five to ten liters using a hollow fiber dialysis ultrafiltration system (Amicon Model DC30; Amicon Corp., Lexington, Mass.).

The upper ten centimeters of the sediment was sampled with a non-aseptic petite Ponar grab. The sediment samples were aseptically subsampled and appropriately diluted for microbiological examination.

Methods employed for measurement of temperature, dissolved oxygen, salinity, and transparency have been reported elsewhere (Sayler et al., 1975a).

3B.2.2 Bacterial Enumeration

A diagram of the microbiological procedures followed in analyzing the samples collected in the study is given in Figure 3B-2. Methods and media employed for determining the total viable bacterial counts (TVC), most probable number of coliforms (MPN) and of fecal coliforms (F-MPN) have been described elsewhere (Sayler et al., 1975a). However, the total viable bacterial counts in samples of 20 percent salinity were determined using Marine Agar 2216 (Difco Laboratories, Detroit, Mich.).

Enumeration of *Salmonella* spp. was attempted using the classical selective enrichment procedure including incubation at elevated temperature (Spino, 1966). Non-selective enrichment also was used. Selective enrichment by *Salmonella* spp. was done using tetrathionate broth (Difco) and selenite cystine broth (Bioquest, Cockeysville, Md.). Triplicate tubes of each medium were inoculated with one milliliter of a one-tenth dilution of bottom sediment. Bottom water samples (100 ml) were filtered through a 0.45-μm membrane. Additional enrichment broths were inoculated with one milliliter

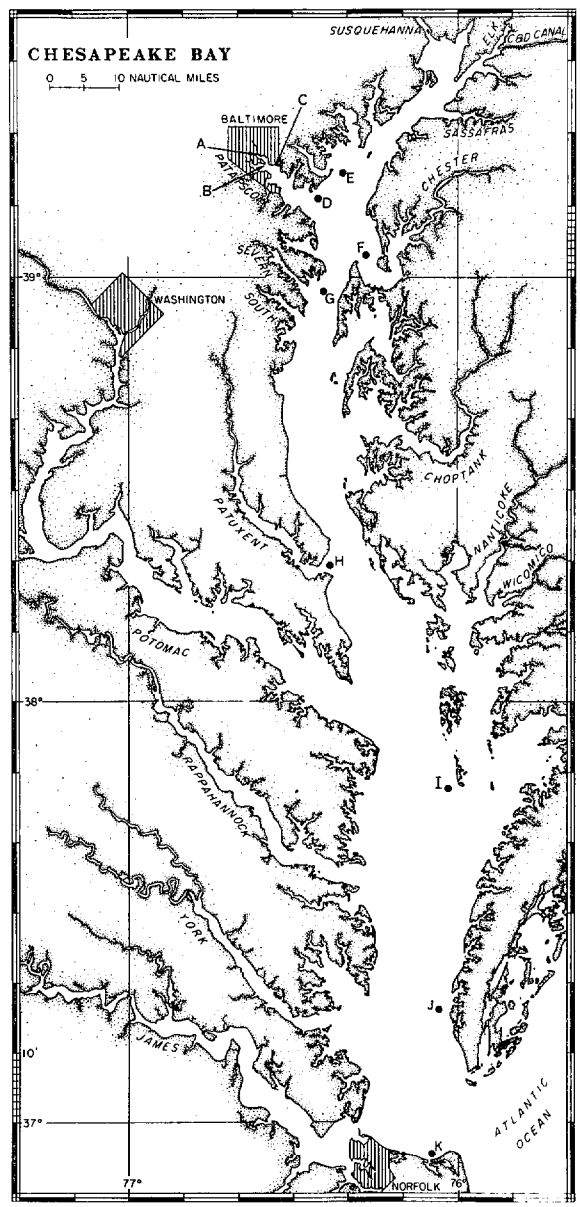

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Figure 3B-1. Chesapeake Bay Stations Sampled in the Study of March–June, 1975

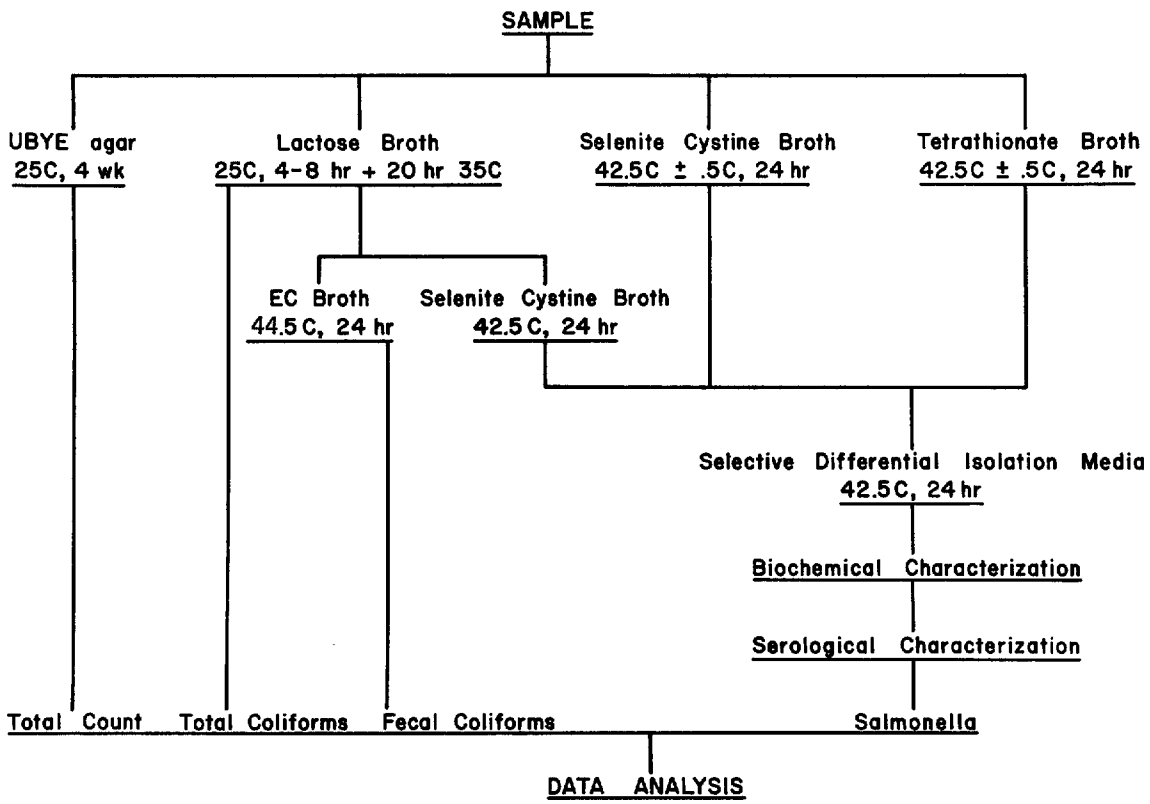

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Figure 3B-2. Procedure for Enumeration and Identification of Salmonella Employing Non-Selective Enrichment

of the dialysis concentrate of the surface water samples. Inoculated selective enrichment media were immediately placed in a constant temperature water bath and incubated at 42.5°C for 24 hours. Incubation up to 48 hours in selenite cystine and tetrathionate broth was unsuccessful for recovery of confirmed *Salmonella* spp. (Sayler et al., 1975b).

Non-selective enrichment for *Salmonella* was achieved by inoculation of nine replicate lactose broth (Difco) tubes in triplicate, using 0.1 ml, 1.0 ml and 10.0 ml volumes of bottom water, surface water concentrate, or sediment dilution. The inoculated tubes were allowed to incubate at ambient temperature (about 25°C) for approximately four hours, followed by incubation at 35°C for a combined total incubation time of 24 to 48 hours. Immediately after non-selective enrichment, the lactose broth tube cultures were transferred to EC broth (Difco) and selenite cystine broth and incubated 24 hours at 44.5 ± .5°C and 42.5 ± .5°C, respectively. Thereby, fecal coliform and *Salmonella* enumeration, respectively, were calculated from tubes showing confirmed growth in the medium.

Following non-selective and selective enrichment, the selenite cystine and tetrathionate broth cultures were streaked onto Bismuth Sulfite Agar (Difco) and the inoculated plates were incubated at 35°C for 24 to 48 hours. Isolated colonies were purified and maintained on Nutrient Agar (Difco) (Sayler et al., 1975a) or on Trypticase Soy Agar (BioQuest).

3B2.3 Characterization of Isolates

All of the pure cultures were biochemically characterized using the API 20 system (Analytab Products Inc., Plainview, N. Y.), and they were identified to species using the API computerized profile register. Confirmation of each of the strains of *Salmonella* was done serologically at the Center for Disease Control (CDC), Atlanta, Georgia.

3B3. RESULTS AND DISCUSSION

3B.3.1 Recovery of Salmonella

A total of 34 samples was collected at the 11 Chesapeake Bay sampling sites and four (11.8 percent) were found to contain *Salmonella* spp. As shown in Table 3B-1, all strains of *Salmonella* were recovered using the non-selective enrichment method. Also, all of the isolates were obtained at a single sampling station. Significantly the concurrent selective enrichment method, which employs tetrathionate or selenite broth, resulted in no presumptive or confirmed *Salmonella* spp. isolation.

A total of 12 *Salmonella* strains were obtained, including 11 *Salmonella enteritidis,* serotype *derby* and one *Salmonella enteritidis,* serotype *infantis.* A presumptive *Salmonella* was concluded to have been misidentified and was biochemically and serologically confirmed as *Klebsiella pneumoniae,* Type 45. Of approximately 1300 known serotypes, the two *Salmonella enteritidis* serotypes are among the 12 most frequently encountered from human and non-human specimens (Martin and Ewing, 1969).

The 12 *Salmonella enteritidis* strains were isolated from samples collected at Jones Falls (Station A in Figure 3B-1) in Baltimore harbor. Compared with the other stations sampled (Table 3B-2), Jones Falls is a shallow, low salinity site located in the northwest branch of Baltimore harbor. It is located at the confluence of Jones Falls, a stream carrying sewage treatment effluent, and Baltimore harbor. Extending outward to the Chesapeake Bay proper, the water was progressively brackish and of a greater depth, although not necessarily containing fewer coliforms. There was no significant ($\alpha = 0.05$) correlation of *Salmonella* recovery with either transparency, temperature, dissolved oxygen or TVC. A significant negative correlation ($r = -0.45$) at the 90 percent probability level was observed between salinity and *Salmonella* recovery using the non-selective enrichment technique.

Salmonella spp. were recovered with greater frequency from sediment, although they were recovered from both bottom water and the concentrated surface water samples (Tables 3B-2 and 3B-3). A significant correlation was observed for fecal coliform MPN and presence of *Salmonella enteritidis* ($r = 0.65$, $P = 0.95$) in both water and sediment samples. Assuming the presence of *Salmonella* spp. to be related to incidence of fecal coliforms, a regression equation ($Y = 0.002X + 0.006$, $r^2 = 0.42$) was computed from the data given in Tables 3B-2 and 3B-3. It was estimated that one *Salmonella* would be recovered for every 497 fecal coliforms. This value is comparable to other estimates of fecal coliform densities related to *Salmonella* recovery (Lear and Jaworski, 1969; Spino, 1966; Van Donsel and Gelreich, 1971). Quantitative estimates of the incidence of *Salmonella* spp. computed for the data from the non-selective primary enrichment MPN, indicated *Salmonella* levels ranging from zero to 9.1 per 100 ml in the water and from 3.6 to 36 per 100 g in the sediment (Table 3B-3). Van Donsel and Geldreich (1971) have previously reported elevated *Salmonella* counts in sediment to be higher, compared with water, but at a ratio of 14,000 fecal coliforms to one *Salmonella,* a ratio much different from the ratio of 10 to 30 fecal coliforms to one *Salmonella* observed in this study (Table 3B-3).

TABLE 3B-1. RECOVERY OF SALMONELLA FROM CHESAPEAKE BAY SAMPLES EMPLOYING NON–SELECTIVE AND SELECTIVE ENRICHMENT

Source		Confirmed* *Salmonella* Enrichment Method			
		Tetrathionate	Selenite	Lactose–Selenite	Percent
Samples	(34)	0	0	4	11.8
Sampling Sites	(11)	0	0	1	9.1

*Biochemical and serological confirmation

TABLE 3B-2. PHYSICAL, CHEMICAL, AND MICROBIOLOGICAL CHARACTERISTICS OF CHESAPEAKE BAY SAMPLES FROM WHICH RECOVERY OF CONFIRMED SALMONELLA WAS OBTAINED

	Station	Sample Type	Depth* (m)	Salinity (°/oo)	Dissolved Oxygen (mg/1)	Transparency (m)	Temperature (°C)	TVC	Coliform MPN	Fecal Coliform MPN	*Salmonella*
A	Jones Falls**	W	2.6	1.4	6.9	0.6	22.6	1.8×10^6	> 1,100	738	3
		S						1.9×10^7	> 1,100	745	9
B	Fort McHenry†	W	6.5	3.9	10.3	0.7	21.1	5.9×10^6	> 1,100	132	0
		S						1.1×10^7	> 1,100	571	0
C	Colgate Creek†	W	9.1	3.9	8.5	0.8	22.5	1.4×10^6	> 1,100	93	0
		S						1.7×10^7	> 1,100	20	0
D	Fort Howard†	W	4.5	3.2	6.8	2.0	18.6	1.6×10^5	240	0	0
		S						7.1×10^6	> 1,100	0	0
E	Middle River†	W	5.7	0.6	9.1	1.0	19.5	8.3×10^4	--	--	0
		S						8.0×10^5	--	--	0
F	Chester River††	W	10.0	8.6	9.5	2.0	14.5	6.0×10^4	14	0	0
		S						3.1×10^6	460	0	0
G	Tolley Bar††	W	6.5	7.0	6.2	1.8	15.1	2.0×10^5	3.6	0	0
		S						3.2×10^6	> 1,100	3.6	0
H	Solomons††	W	9.7	10.9	8.5	1.7	23.0	1.8×10^4	460	3.6	0
		S						4.1×10^6	210	0	0
I	Tangier Island††	W	6.1	14.0	3.6	2.3	23.0	5.0×10^4	23	0	0
		S						2.9×10^6	9.1	0	0
J	Cape Charles††	W	28.0	25.8	7.4	3.3	--	2.2×10^5	240	0	0
		S						2.3×10^6	240	0	0
K	Little Creek††	W	8.2	20.3	5.8	1.7	25.4	3.3×10^4	9.1	0	0
		S						1.5×10^7	1,100	9.1	0

* Samples collected 1.0 meter above the sediment–water interface.
** Samples collected in March, May, and June 1975; mean value given.
† Samples collected in March and May 1975; mean value given.
†† One sample.

TABLE 3B-3. RECOVERY OF SALMONELLA ENTERITIDIS FROM SAMPLES OF WATER AND SEDIMENT COLLECTED AT JONES FALLS STATION BY THE NON–SELECTIVE ENRICHMENT PROCEDURE

Sample Type	Date	Coliform MPN	Fecal Coliform MPN	Number of *Salmonella*	*Salmonella* MPN
Water*	5/75	>1,100	1,100	1	3.6
	6/75	>1,100	15	0	0
Sediment**	5/75	>1,100	> 1,100	8	36.0
	6/75	>1,100	35	1	3.6
Dialysis Concentrate***	5/75	>1,100	1,100	2	9.1
	6/75	—	—	—	—

* Bottom water collected one meter above the sediment-water interface.

** Sediment grab sample collected from the upper 10 cm of the sediment.

*** 40-fold concentration of a surface water sample.

3B.3.2 Evaluation of Enrichment Techniques

The enumeration of *Salmonella* employing highly selective enrichment and plating media with elevated temperature (42.5°C) is a recognized standard method (APHA, 1971). There is the tendency to apply this procedure for enumeration of pathogens to all types and classes of water. The initial objective in the use of selective temperature and medium was to enumerate *Salmonella* in samples heavily contaminated with both potential pathogens and related enterics (Spino, 1966; Harvey and Price, 1968). Harvey and Price (1968) stated that a lower temperature of incubation may be required for samples containing smaller populations of *Salmonella* or organisms in need of resuscitation. In addition, Thomson (1955) noted that non-selective nutrient broth enrichment for *Salmonella typhimurium* was equally efficient as selenite broth. Isenberg et al. (1969) found that a variety of selective plating media was equally suited for isolation of *Salmonella* from mixtures of fecal bacteria when employing an incubation temperature of 37°C.

When applying the selective or non-selective enrichment method for isolating *Salmonella* from estuarine samples, one must consider the physical and chemical characteristics of the environment as well as the residence time of the organisms in the environment. Heat resistance of *Salmonella* incubated at temperatures less than 35°C is significantly less than that of *Salmonella* recovered from warmer environments (Ng et al., 1969; Parker-Baird et al., 1970). Furthermore, stationary cells appear to be more resistant to elevated temperature than younger cells (Ng et al., 1969). Parker-Baird et al., (1970) showed decreased heat tolerance with increased salinity. Ng et al., (1969) reported that carbon-limited cells may be more heat resistant than nitrogen-limited cells.

All these factors may have direct influence on enumeration of *Salmonella* from extra-intestinal sources. *Salmonella* may be poor competitors in the aquatic environment (Hendricks, 1972) and enumeration of these organisms may be dependent upon factors that have little effect upon clinical or food isolates.

From the data on recovery of *Salmonella enteritidis* (Tables 3B-1 and 3B-4), it is concluded that a gradual, step-wise increase in temperature of incubation and the use of a non-selective primary enrichment should be employed for enumeration of environmentally stressed *Salmonella*.

It is of interest to note that a greater number of enterics and related organisms was also recovered with the *Salmonella* (Table 3B-4). However, these were not a serious interference, considering that neither of the other selective enrichment methods was able to detect the presence of *Salmonella.*

Approximately seven percent of the cultures isolated using the non-selective enrichment methods were confirmed as *Salmonella* spp. Of the total of 246 cultures collected and examined, five percent (i.e., one of 20) were confirmed to be *Salmonella* spp. In an earlier study employing a viarety of selective media and enrichment temperatures, it was found that four of 179 cultures examined could be tentatively classified as *Salmonella* species. However, none of these was confirmed serologically (Sayler et al., 1975b).

Of significance also was that several other potential pathogens were enumerated and isolated using the non-selective enrichment procedure. These included *Klebsiella* spp., *Pseudomonas aerginosa, Yersinia enterocolitica,* and *Pasteurella multocida.* Thus, it is apparent that the non-selective enrichment techniques may be extremely valuable in assessing the occurrence of a variety of pathogens in the estuarine environment.

3B.3.3 Salmonella Characterization

The API 20 Enterobacteriaceae identification system was found to be well-suited for application in this estuarine microbiology study. As seen from the data given in Table 3B-5, there was a significant variation in the biochemical characteristics of strains serologically identical. In accordance with accepted biochemical tests, Culture No. 42 would have been discarded because of the production of indole. Similarly, Cultures No. 23 and 57 would have been suspect because of acetoin production. All but two of the *Salmonella enteritidis* isolates were unable to decarboxylate lysine. These observations confirm the position taken by Martin and Ewing (1969) that all suspected *Salmonella* should be thoroughly characterized, both biochemically and serologically. No single isolate matched exactly the reference set

TABLE 3B-4. COMPARATIVE RECOVERY OF SALMONELLA AND RELATED ENTERIC BACTERIA USING THE NON–SELECTIVE ENRICHMENT PROCEDURES

Identified Isolates*	ENRICHMENT METHOD						Total	
	Tetrathionate**		Selenite***		Lactose-Selenite****		Recovered	Percent
	Number Recovered	Percent of Total	Number Recovered	Percent of Total	Number Recovered	Percent of Total		
Klebsiella pneumoniae	5	20.0	26	44.1	50	30.7	81	32.1
K. rhinoscleromatis	0	0.0	3	5.1	0	0.0	3	1.2
K. ozaenae	0	0.0	1	1.7	2	1.2	3	1.2
Enterobacter cloacae	1	4.0	3	5.1	26	16.0	30	12.2
E. aerogenes	1	4.0	0	0.0	6	3.6	7	2.8
E. agglomerans	0	0.0	0	0.0	3	1.8	3	1.2
Pseudomonas aeruginosa	1	4.0	1	2.0	21	31.3	23	9.3
P. fluorescens	0	0.0	0	0.0	1	6.0	1	0.4
P. putrefaciens	0	0.0	0	0.0	1	0.6	1	0.4
Proteus mirabilis	12	48.0	3	5.1	0	0.0	15	6.1
P. morganii	0	0.0	0	0.0	1	0.6	1	0.4
P. rettgeri	0	0.0	0	0.0	1	0.6	1	0.4
P. stuartii	3	12.0	3	5.1	0	0.0	6	2.4
Citrobacter freundii	1	4.0	14	23.7	9	5.5	24	9.7
Salmonella enteritidis	0	0.0	0	0.0	12	7.2	12	4.8
Escherichia coli	0	0.0	0	0.0	5	3.1	5	2.0
Yersinia enterocolitica	0	0.0	0	0.0	2	1.2	2	0.8
Serratia marcescens	0	0.0	0	0.0	2	1.2	2	0.8
S. liquefaciens	0	0.0	0	0.0	1	0.6	1	0.4
Pasteurells multocida	0	0.0	0	0.0	1	0.6	1	0.4
Misc. spp.								
Petobacterium	0	0.0	1	1.7	0	0.0	1	0.4
Erwinia	0	0.0	0	0.0	1	0.6	1	0.4
Alcaligenes	0	0.0	0	0.0	2	1.2	2	0.8
Flavobacterium	0	0.0	0	0.0	2	1.2	2	0.8
Unidentified	1	4.0	4	6.8	16	9.8	21	8.5
Total	25		59		163		246	

*Preliminary identification employing API 20 and computer profile register. *Salmonella enteritidis* identification was confirmed serologically.

**18 to 24-hour incubation at 42.5°C in tetrathionate broth.

***18 to 24-hour incubation at 42.5°C in selenite-cystine broth.

****4 to 8-hour incubation at ambient temperature in lactose broth, followed by an addition 14 to 20-hours at 35°C and transfer to selenite cystine broth with incubation at 42.5°C for 18 to 24-hours.

TABLE 3B-5. COMPARISON OF BIOCHEMICAL CHARACTERISTICS OF SALMONELLA ENTERITIDIS ISOLATED FROM ESTUARINE SAMPLES WITH THOSE OF CLINICAL ISOLATES

Test or Substrate	*Salmonella enteritidis* Strain No.												Observed Reaction		Reference Reaction*	
	23	36	42	50	51	52	53	55A	55B	57	61	128	Sign**	Mean Percent +, (+)	Sign	Percent +, (+)
ONPG	–	–	–	–	–	–	–	–	–	–	–	–	–	0	–	0
Arginine dehydrolase	+	+	–	–	–	–	–	–	–	–	+	+	+	33.3	+ (+)	92.8
Lysine decarboxylase	–	–	–	–	–	–	–	–	–	–	+	+	+	16.7	+	94.9
Ornithine decarboxylase	–	+	+	+	+	+	+	+	+	+	+	+	+	91.7	+	96.7
Citrate	+	+	+	+	+	–	+	–	–	–	–	–	+	50.0	+ (+)***	90.8
H_2S	+	+	+	+	+	–	+	–	+	–	–	+	+	66.7	+	93.7
Urease	–	–	–	–	–	–	–	–	–	–	–	–	–	0	–	0
Tryptophane deaminase	–	–	–	–	–	–	–	–	–	–	–	–	–	0	–	0
Indole	–	+	–	–	–	–	–	–	–	–	–	–	–	8.3	–	0
Voges–Proskauer	+	–	–	–	–	–	–	–	+	–	–	–	+	16.7	–	0
Gelatin	–	+	–	–	–	–	–	–	–	–	–	–	–	8.3	–	0
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	100.0	+	100.0
Mannitol	–	–	+	+	+	+	+	+	+	+	+	+	+	83.3	+	100.0
Inositol	–	–	+	+	+	+	+	+	+	+	+	–	+	75.0	+ (+) –	39.1
Sorbitol	–	–	+	+	+	+	+	+	+	+	+	+	+	83.3	+	97.9
Rhamnose	–	–	+	+	+	+	+	+	+	+	+	+	+	83.3	+	95.2
Sucrose	–	–	–	–	–	–	–	–	–	–	–	–	–	0	–****	0
Melibiose	–	–	+	+	+	+	+	+	+	+	+	+	+	83.3	+****	91.6
Amygdaline	–	–	–	–	–	–	–	–	–	–	–	–	–	0	–****	0
Arabinose	–	–	+	+	+	+	+	+	+	+	+	+	+	83.3	+	99.4
Nitrate	+	+	+	+	+	+	+	+	+	+	+	+	+	100.0	+	100.0
Oxidase	–	–	–	–	–	–	–	–	–	–	–	–	–	0	–	0

*Edwards and Ewing (3)
**Greater than ten percent recorded as +
***Positive after three days
****API reference

of biochemical reactions for *Samonella enteritidis* as given by Edward and Ewing (1972). However, the overall observed mean (occurrences greater than ten percent recordered as + observation) exactly matched the reference reaction, except for the (16.7%+) Voges-Proskauer reaction.

The API profile register facilitated the correct identification of the unknown isolates. Identification in this study was based on the probability of occurrence and was not dependent upon a specific reaction or reaction pattern.

Smith et al., (1972) reported 100 percent accuracy for the identification of *Salmonella* spp. and a 96.4 percent overall accuracy when the API 20 Enterobacteriaceae test system was employed. Washington et al., (1971) demonstrated greater than 90 percent accuracy for a number of Enterobacteriaceae. Twelve of 13 *Salmonella* isolates obtained in this investigation were serologically confirmed, following biochemical identification, which resulted in a net accuracy of 92.3 percent for *Salmonella enteritidis.* As indicated by other investigators, the manufacturer's instructions must be closely followed in order to achieve accurate results. It should be pointed out that all isolates obtained when using selective and non-selective enrichment procedures were tested in an identical manner. Therefore the recovery of *Salmonella,* employing the non-selective enrichment procedure, was not an artifact of the identification procedure.

It is concluded from this study that a non-selective primary enrichment medium, when used in conjunction with a stepwise increase in temperature of incubation, is efficient in isolating *Salmonella* and other pathogens frequently missed when standard, recommended selective enrichment and elevated temperature enumeration methods are employed. It is necessary, however, to accept a certain degree of interference by other enteric bacteria because of the less stringent enrichment conditions. Therefore, a large number of organisms often must be screened and the identification will require the use of a probability matrix for frequency of occurrence of characteristics, rather than a monothetic classification based on selected biochemical reactions. Clearly, the presence of a potential public health hazard otherwise would not be detected.

3B4. ACKNOWLEDGEMENTS

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CHAPTER 4

ESTUARINE SEDIMENTOLOGY

4.1 Bottom Sediments

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4.2 Suspended Sediments

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ABSTRACT

Analyses of both suspended and bottom sediments recovered during the course of the Upper Bay Survey support the contention that the finer grain-sizes of natural origin carry the highest concentrations of chlorinated hydrocarbons. The origins of these materials include internal (generally biogenic) sources, marginal contributions from shoreline erosion, and external sources contributed by fluviatile agents, especially those associated with high volume runoff accompanying the spring freshet. The fine-grained bottom deposits located in the central part of the Chesapeake Bay form a sink for chlorinated hydrocarbons. The suspended load is localized in the turbidity maximum occupying a region some 30 km long upstream from Tolchester.

During 1974, the estimated yield of sediment from the Susquehanna River, the major source of water and suspended sediment in the upper bay, was 0.8×10^6 metric tons, a value some 20 percent lower than the averages obtained over several years of record. Much of the suspended sediment particles recovered may be considered agglomerates produced by biological activity. Electrostatic flocculation appears to play a minor role in the formation of agglomerates, and biogenic precipitation is considered the primary depositional agent for the fine-grained materials in suspension.

TABLE OF CONTENTS

Paragraph		Page
4.	ESTUARINE SEDIMENTOLOGY	4-1
4.1	Bottom Sediments	4-1
4.1.1	Background	4-1
4.1.1.1	Physiographic and Geologic Setting	4-1
4.1.1.2	Previous Work	4-2
4.1.2	Techniques	4-5
4.1.2.1	Field Techniques	4-5
4.1.2.2	Laboratory Techniques	4-5
4.1.3	Sedimentology	4-5
4.1.3.1	Bottom Sediments	4-5
4.1.4	Discussion	4-17
4.1.5	Summary	4-25
4.1.6	Acknowledgements	4-31
4.1.7	References	4-31
4.2	Suspended Sediment in the Waters of the Upper Chesapeake Bay	4-34
4.2.1	Introduction	4-34
4.2.2	Sources of Sediment	4-34
4.2.3	Suspended Sediment Population	4-41
4.2.4	Distributions of Temperature, Salinity, and Total Suspended Solids	4-41
4.2.4.1	Temperature and Salinity	4-41
4.2.4.2	Total Suspended Solids	4-41
4.2.5	Size Distribution of Suspended Particles	4-42
4.2.5.1	Methods	4-42
4.2.5.2	Results	4-47
4.2.5.3	Discussion	4-59
4.2.6	Acknowledgements	4-62
4.2.7	References	4-62

LIST OF FIGURES

Figure		Page
4-1	Bathymetric Chart of the Upper Chesapeake Bay	4-3
4-2	Generalized Geologic Map of the Upper Chesapeake Bay and Bottom Sample Station Locations	4-4
4-3	Sample Record from Coulter Counter Analyses	4-6
4-4	Distribution of Sand in the Upper Chesapeake Bay	4-7
4-5	The Silt-Sand Ratios in the Upper Chesapeake Bay	4-8
4-6	Percent Clay in Bottom Sediments	4-9
4-7	The Three Sources of Sediment to the Bay	4-11
4-8	Satellite (ERTS-1) Photograph of the Upper Chesapeake Bay, April 3, 1974	4-12
4-9	Scanning Electron Microscope Images of Quartz Silt	4-13

LIST OF FIGURES (Continued)

Figure		Page
4-10	Distribution of Quartz Silt in Bottom Sediments	4-14
4-11	Scanning Electron Microscope Image of Mica (Muscovite) from Bay Sediments	4-15
4-12	Distribution of Mica in Bay Sediments	4-16
4-13	Scanning Electron Microscope Image of Glauconite Grain	4-18
4-14	Distribution of Glauconite in Bay Sediments	4-19
4-15	Scanning Electron Microscope Images of Slag Fragments in Upper Bay Bottom Sediments	4-20
4-16	Distribution of Slag and Coal in Bottom Sediments	4-21
4-17	Diatom Frustule Comprising Some of the Biogenic Debris in Bottom Sediments of the Upper Bay	4-22
4-18	Triangle Diagram for Displaying Sediment Size Characteristics	4-23
4-19	Pattern of Sample Distribution for Bedload Materials and Pattern of Sample Distribution for Deposits Resulting from Suspended Sediment	4-23
4-20	Triangle Diagram for Bottom Samples Recovered During the Upper Bay Survey	4-24
4-21	Comparison of Mean Grain Size, in Phi Units	4-26
4-22	Comparison of Mean Grain Size, in Phi Units	4-27
4-23	Comparison of Mean Grain Size, in Phi Notation	4-28
4-24	Chlordane Concentrations in Bottom Sediments from Baltimore Harbor and the Chester River	4-29
4-25	Polychlorinated Biphenyl Concentrations in Bottom Sediments from Baltimore Harbor and the Chester River	4-30
4-26	DDT Residue Concentrations in Bottom Sediments from Baltimore Harbor and the Chester River	4-30
4-27	Ensemble Average of the Susquehanna River at Conowingo, 1929-1966	4-35
4-28	Discharge of the Susquehanna River at Conowingo in 1973	4-37
4-29	Discharge of the Susquehanna River at Conowingo in 1974	4-37
4-30	Concentration (mg/l) of Suspended Sediment in the Susquehanna River During 1973	4-38
4-31	Concentration (mg/l) of Suspended Sediment in the Susquehanna River During 1974	4-38
4-32	Suspended Sediment Discharge of the Susquehanna River at Conowingo During 1973	4-39
4-33	Suspended Sediment Discharge of the Susquehanna River at Conowingo During 1974	4-39
4-34	Photomicrograph of Sample of Suspended Sediment from Upper Chesapeake Bay	4-44
4-35	Photomicrograph of Sample of Suspended Sediment from Upper Chesapeake Bay	4-45
4-36	Photomicrograph of Sample of Suspended Sediment from Upper Chesapeake Bay	4-46
4-37	Photomicrographically Determined Number-Size Distributions of Suspended Sediment	4-48
4-38	Photomicrographically Determined Number-Size Distributions of Suspended Sediment	4-48
4-39	Histograms of the Mean Sizes of All Samples	4-56
4-40	Histograms of the Modal Class of Equivalent Projected Diameters	4-57
4-41	Histogram of the Modal Class of Equivalent Projected Diameters	4-58

LIST OF TABLES

Table		Page
4-1	Statistical Properties of the Number-Size Distributions of the Suspended Particle Population of the Upper Chesapeake Bay	4-49
4-2	Statistical Properties of Volume-Settling Velocity Distributions of Samples of Suspended Sediment from Upper Chesapeake Bay	4-60

4. ESTUARINE SEDIMENTOLOGY

4.1 Bottom Sediments

4.1.1 Background

4.1.1.1 Physiographic and Geologic Setting

The Chesapeake Bay is recognized as one of the largest estuaries in the world, and within the United States it is the longest (290 km) and second largest (11,390 km^2) estuarine system. This report is focused upon the geological and sedimentological relationships present in what will be referred to as the upper Chesapeake Bay. This portion of the bay lies north of the confluence of the Severn River and the bay proper, and discussion will be limited to studies performed in that area.

The dendritic form of the upper Chesapeake Bay is inherited from the drowned tributary network which fed the ancestral Susquehanna River during the most recent (Holocene) period of depressed sea level (Hack, 1957). Earlier courses for this major east coast river are speculative (Hansen, 1966; Weaver and Hansen, 1966), and recent but controversial interpretations of aeromagnetic anomalies suggest that the upper Chesapeake Bay orientation may reflect early and persistent conformity to a deep fault-bounded basin (Higgins and others, 1974a, 1974b; Hansen, 1974).

Relief in the coastal plain bordering the upper bay is subdued, and the most prominent positive features are the low terrace scarps which face the bay. The origin of these erosional features is not clear, but several studies (Schlee, 1957) suggest that those terraces higher than 30 meters may be ascribed to fluviatile agents, whereas those at lower elevations may be marine in origin. Discussion of these features is cited in Palmer (1972), and new evidence supplied by DeAlteras (1975) and Hicks (1972) provides additional argument for regional uplift and subsidence, respectively, in the central Atlantic coastal plain.

The units forming the physical margins of the upper bay are soft, unconsolidated to weakly cemented Cretaceous and Tertiary sandstones, siltstones, and similar detrital deposits which are subject to accelerated shoreline erosion as a result of nearshore processes (Schubel and others, 1972) and local groundwater conditions (Palmer, 1973). Their contribution to upper Chesapeake Bay sedimentary processes will be discussed later. Much of the bay's margin consists of wetland areas whose dominant flora, Spartina, tends to bind and retain soft materials. However, exposed beach and bluff areas are prone to erosion produced by a combination of elements acting from both the bay and

onshore. Conservative estimates of property loss in this area account for scores of acres per year which are lost to the bay, and similar figures are quoted for other regions within Chesapeake Bay.

Much of the upper Chesapeake Bay consists of shoal areas marginal to tributaries and/or shorelines faced by bluffs of soft sedimentary units. The general bathymetric configuration of the area is shown in Figure 4-1, and it will be noted that the mean depth is quite shallow — less than four meters. This is a common feature in the shallow estuaries of the Atlantic coastal plain since the present bathymetric character reflects the recent drowning of river and stream courses and a redistribution of shoreline erosional products and beach materials. Thus, extensive shoals bordering the major channel are a reflection of a recent, and at present incomplete, readjustment of a former fluviatile terrain to a drowning event. The tidal flow accompanying such drowning now is redistributing these materials, which in many places within the upper bay derived from shoreline erosion. The main channel (thalweg) of the upper bay is in part an inherited feature and also a result of dredging by the U.S. Army Corps of Engineers. Circulation of upper bay waters is affected by the displacement of the thalweg to the eastern margin of the upper bay, and the effect of this feature on sediment distribution will be examined in a later section.

The structure and stratigraphy in the two regions forming the eastern and western boundaries of the upper bay are profoundly different (Figure 4-2). The western shore consists of a relatively thin veneer of Mesozoic and Cenozic sedimentary units which wedge out against the crystalline Appalachian Province (or Piedmont Province) consisting primarily of Paleozoic metamorphic units which locally surround Precambrian and Paleozoic intrusive rocks. The Cretaceous and younger sedimentary units of the Coastal Plain Province thicken and dip to the southeast, and along the axis of the present Chesapeake Bay, these formations reach a thickness of nearly 800 meters. Along the Atlantic shoreline of the Delmarva Peninusla, the thickness of this clastic wedge reaches at least 2,280 meters (Vokes, 1957) and definite basement (metamorphic rocks similar to Baltimore gabbros) are cored at a depth of 2,180 meters near Berlin in Worcester County, Maryland. A concise description of the geologic framework of this entire region may be found in Maher and Applin (1971).

Sedimentary units of the coastal plain which border the upper bay consist of flat-lying beds of weakly cemented sandstones, siltstones, and clays. Gravel lenses are not uncommon, and in local areas gravels may be sufficiently abundant to form economic concentrations. An examination of Figure 4-2 will reveal most of the shoreline of the Upper Bay consists of Quaternary alluvium. On the basis of mineralogy, fossil content, and elevation, Cleaves and others (1968) have subdivided the Pleistocene of this region into the Upland and Lowland deposits, but for the purpose of this report this distinction has not been made. The weak nature of these formations is a major factor in both shoreline erosion and in the introduction of detrital materials into the Upper Bay. The importance of marginal sedimentary processes will be discussed under a later section.

4.1.1.2 **Previous Work**

A summary of previous geological work in the area of the Upper Bay is provided by Palmer (1972), and it will not be repeated here. However, continuing studies in the sedimentology of the bay have resulted in a number of recent communications which bear upon the origin and distribution of Chesapeake Bay sediments. Gas contained within bay sediments has been found to create acoustically impenetrable zones (Schubel and Schiemer, 1973), a factor which has frustrated many efforts to record high resolution seismic reflection profiles in this area. The upper bay region displays numerous examples of gas-charged sediments.

Sedimentological studies related to the transport of trace metals and chlorinated hydrocarbons on particulate matter have been reported by Palmer (1972), Helz (1974), and Palmer and Munson (1974). Investigations in regional sedimentation are discussed by Palmer and others (1973), Owens and others (1974), Palmer (1974), Nichols (1975), Siegrist (1975), and Merryman and Palmer (1975). The accelerated production of suspended sediment due to urban construction in the Pautuxent River watershed is discussed by Roberts and Pierce (1974), and the role of groundwater in shoreline erosion in the upper Chesapeake Bay is described by Palmer (1973). Many of the agents and processes acting in the upper bay are also present in large tributaries, and the comprehensive Chester River Study summarized by Clarke and others (1972) provides insight into mechanisms which determine transport and depositional processes.

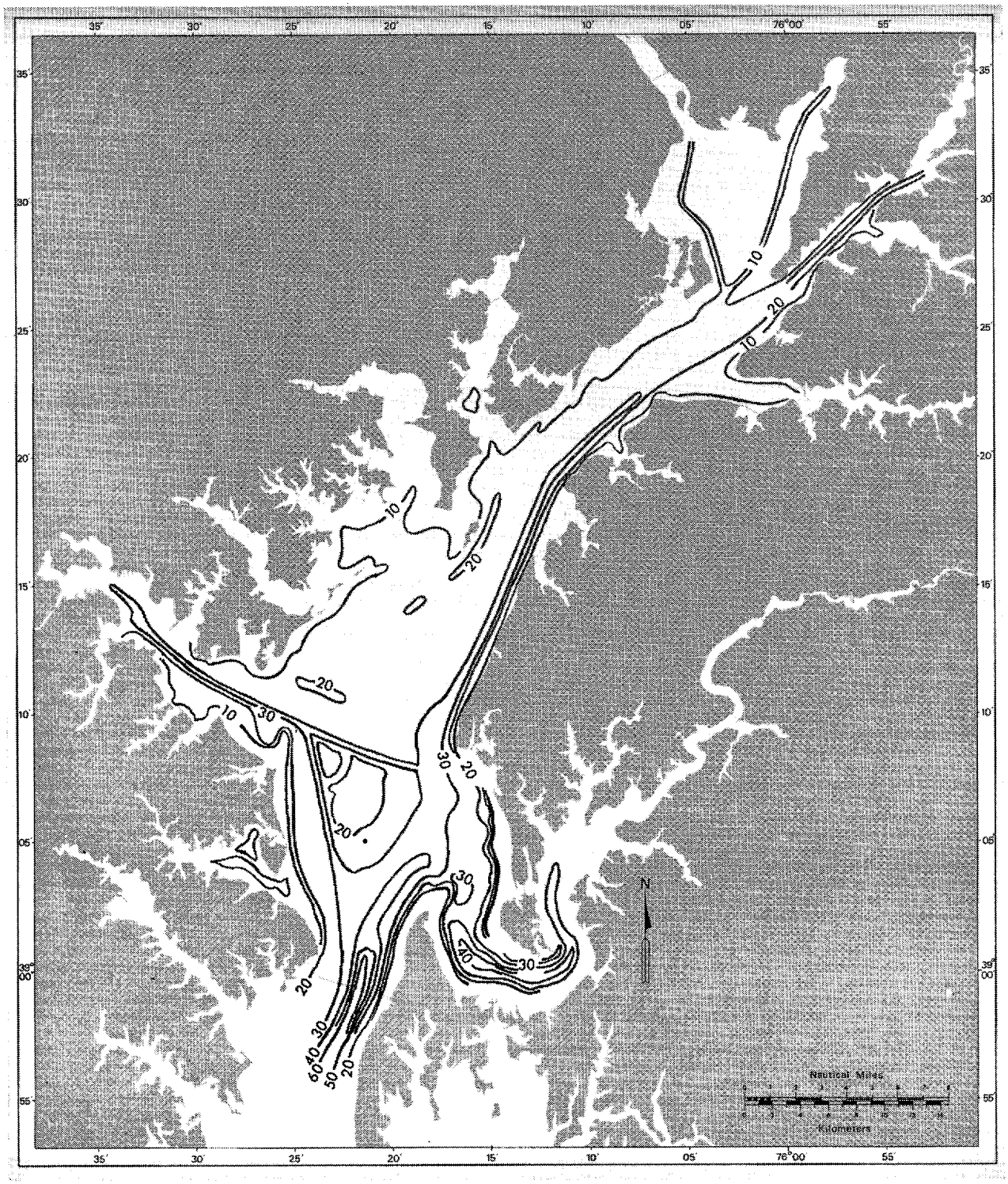

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Figure 4-1. Bathymetric Chart of the Upper Chesapeake Bay (Contour Interval 10 Feet)

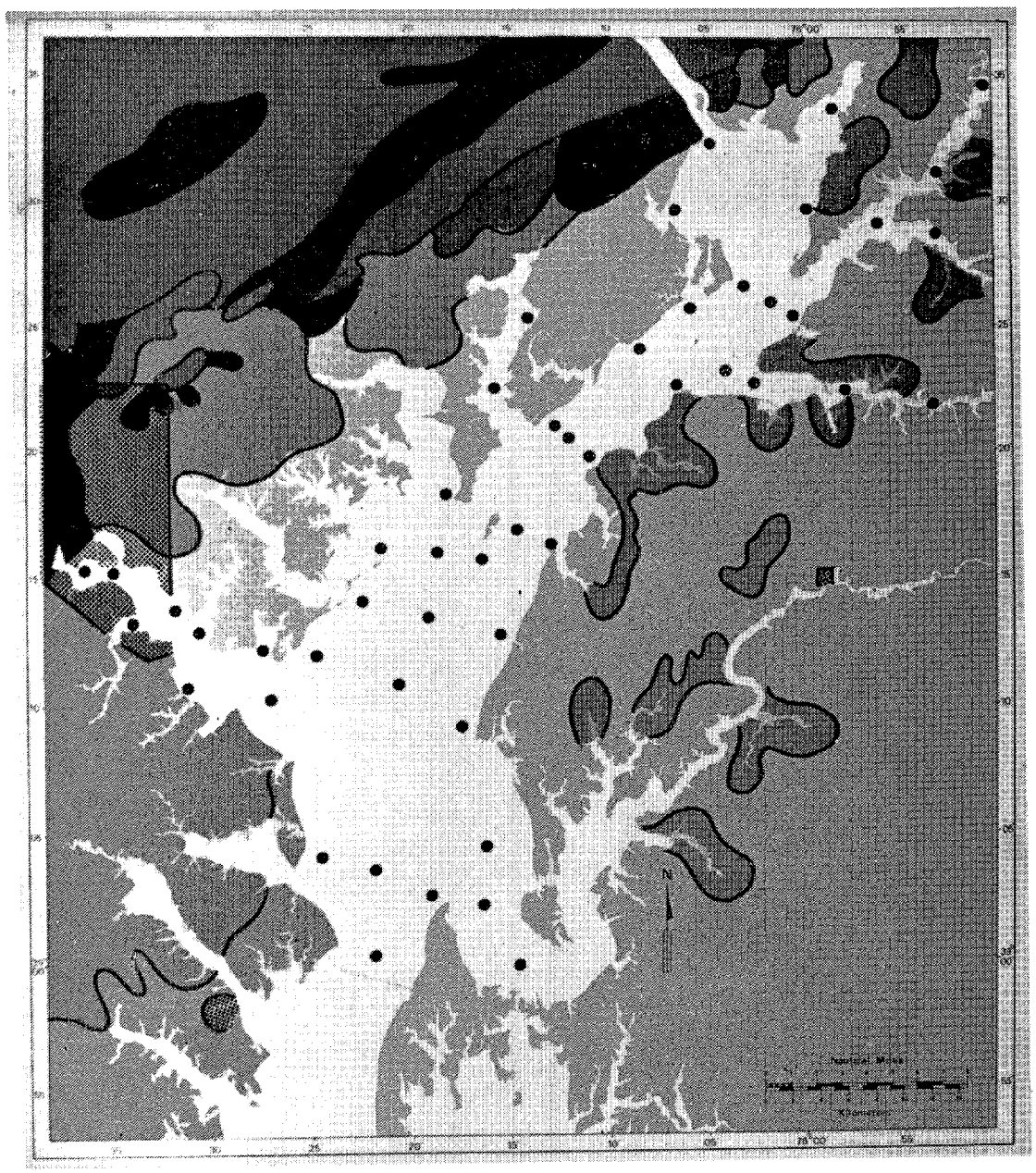

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Figure 4-2. Generalized Geologic Map of the Upper Chesapeake Bay and Bottom Sample Station Locations. (Darkest grey shades are metamorphic and igneous rocks of the Piedmont province. Medium grey is Cretaceous sedimentray rocks, and lightest grey represents Quaternary deposits. Baltimore is shown as stippled area in center left; Annapolis at lower left.)

4.1.2 Techniques

4.1.2.1 Field Techniques

Field techniques employed to collect both bottom and suspended sediment samples followed standard techniques. Bottom samples were recovered with either a Ponar or Ekman grab sampler. Representative portions of the materials to be analyzed for sedimentological parameters were retained in cartons and returned to the laboratory. All samples were analyzed as quickly as possible after recovery.

4.1.2.2 Laboratory Techniques

The initial set of 55 bottom sediment samples were wet-sieved through a 62-micron sieve, and where the coarse fraction (sieve diameter greater than 62 microns) was sufficiently large to warrant analyses by size fraction, the sand fraction was split into whole phi (ϕ) classes. In many cases, standard settling tube routines were not employed because of the high percentage of abnormally light (low specific gravity) sand size particles, and routine sieving techniques were used instead. Much of the sand size material in the fine-grained samples consists of coal (sp. gr. of about 1.5) and slag which contains an abundance of bubbles which yield various specific gravities for individual particles. Fine materials (those passing the 62-micron sieve) removed from the initial set of samples were run by standard pipette analyses (Royse, 1970).

Samples recovered during subsequent cruises were analyzed using the Coulter Electronics particle size analyzer (Model TA) employing apertures of 140, 100, and/or 30 microns. For a description of this technique, see Swift and others (1972). Representative plots from these analyses are presented in Figure 4-3.

Fine-grained sediments from estuaries, rivers, and other non-marine regions often are enriched in organic materials which, because of their propensity for inducing flocculation, make size analyses difficult. A number of analytical techniques have been proposed to deal with such materials. The approach used in this study included soaking the fine fraction (material passing the 62-micron sieve) in a 30 percent hydrogen peroxide solution heated to 50-54°C for periods of several days, and subsequent transfer to a 50 percent acetone-water mixture for final deflocculation. The materials were then transferred to a one-liter graduate and sampled according to standard pipette methods at periods of zero, two, and eight minutes to provide weights for fractions down to 16 microns. The remaining suspensate in the graduate was re-stirred, re-sampled, and passed through a 15-micron mesh fabric sieve to remove slag, coal, and other particles of coarse to medium silt size. Materials passing the 15-micron mesh were then analyzed in the Coulter Counter employing a 30-micron aperture. (Thresholds for maximum particle size detection in the Coulter Counter are generally one-half the aperture diameter, hence the 15-micron mesh provides an upper cutoff for the 30-micron aperture). All size data were combined in a single program which accepts inputs from settling tube analyses, pipette fractions, and the Coulter Counter analyses. These data were then entered in the data base.

4.1.3 Sedimentology

4.1.3.1 Bottom Sediments

The distribution of bottom sediment type in the upper Chesapeake Bay varies markedly over short distances, and at fixed points as well, since the influx of coarser materials to the sedimentary system is often seasonal and a function of the magnitude of the spring freshet from the Susquehanna and lesser tributaries. During this study, the preliminary set of samples provided the basis for preparation of general maps of sediment distribution based upon sand, silt, and clay content (Figure 4-4 thru 4-6). These must be considered approximate representations, because it is economically impossible (and scientifically irrelevant) to sample on a fine grid which would cover all tributaries, small embayments, etc. where sediment texture can vary widely over distances of tens of meters.

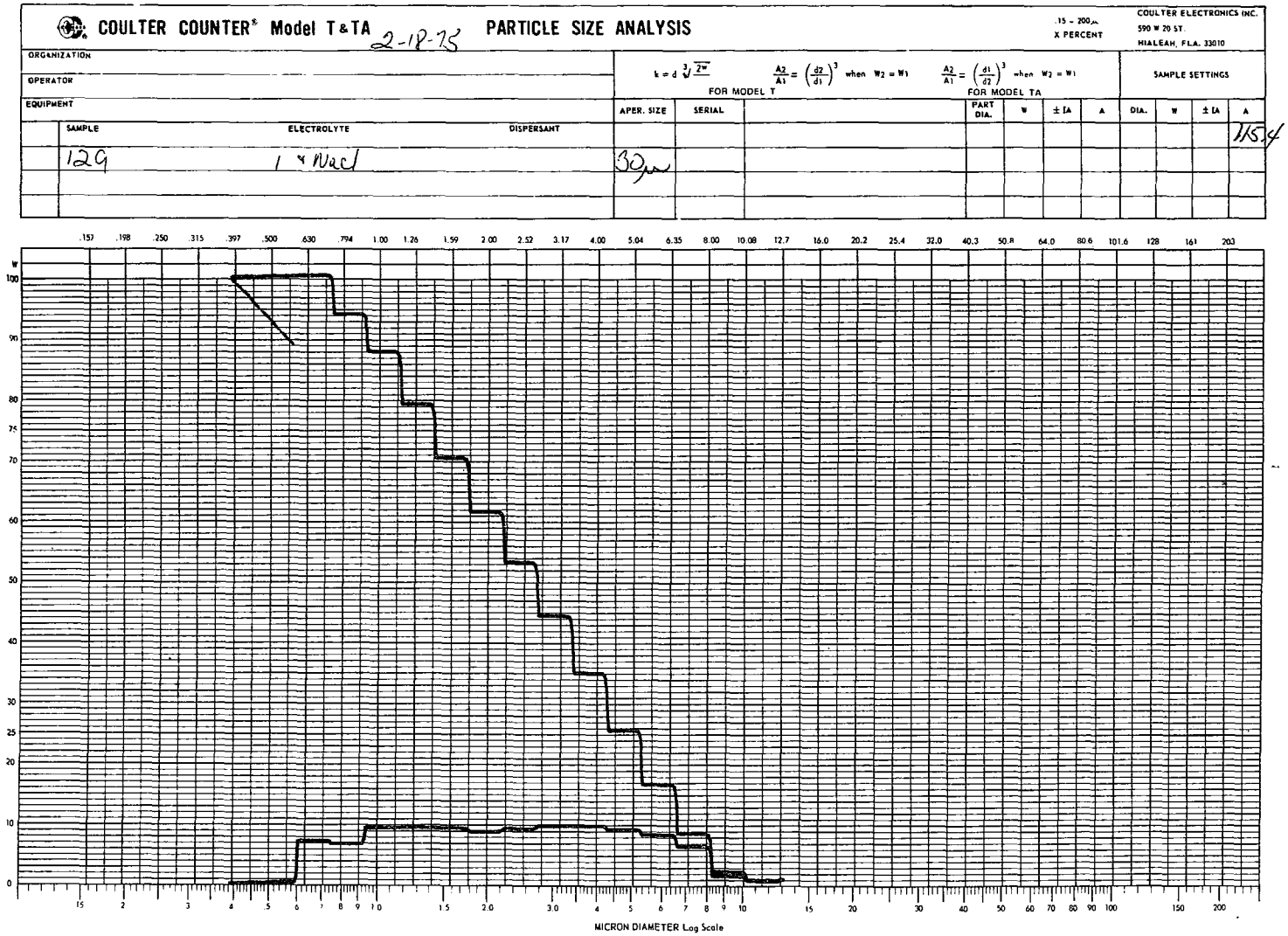

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Figure 4-3. Sample Record from Coulter Counter Analyses. (Both histogram and cumulative frequency curves are presented. Sample is from Station 7A.)

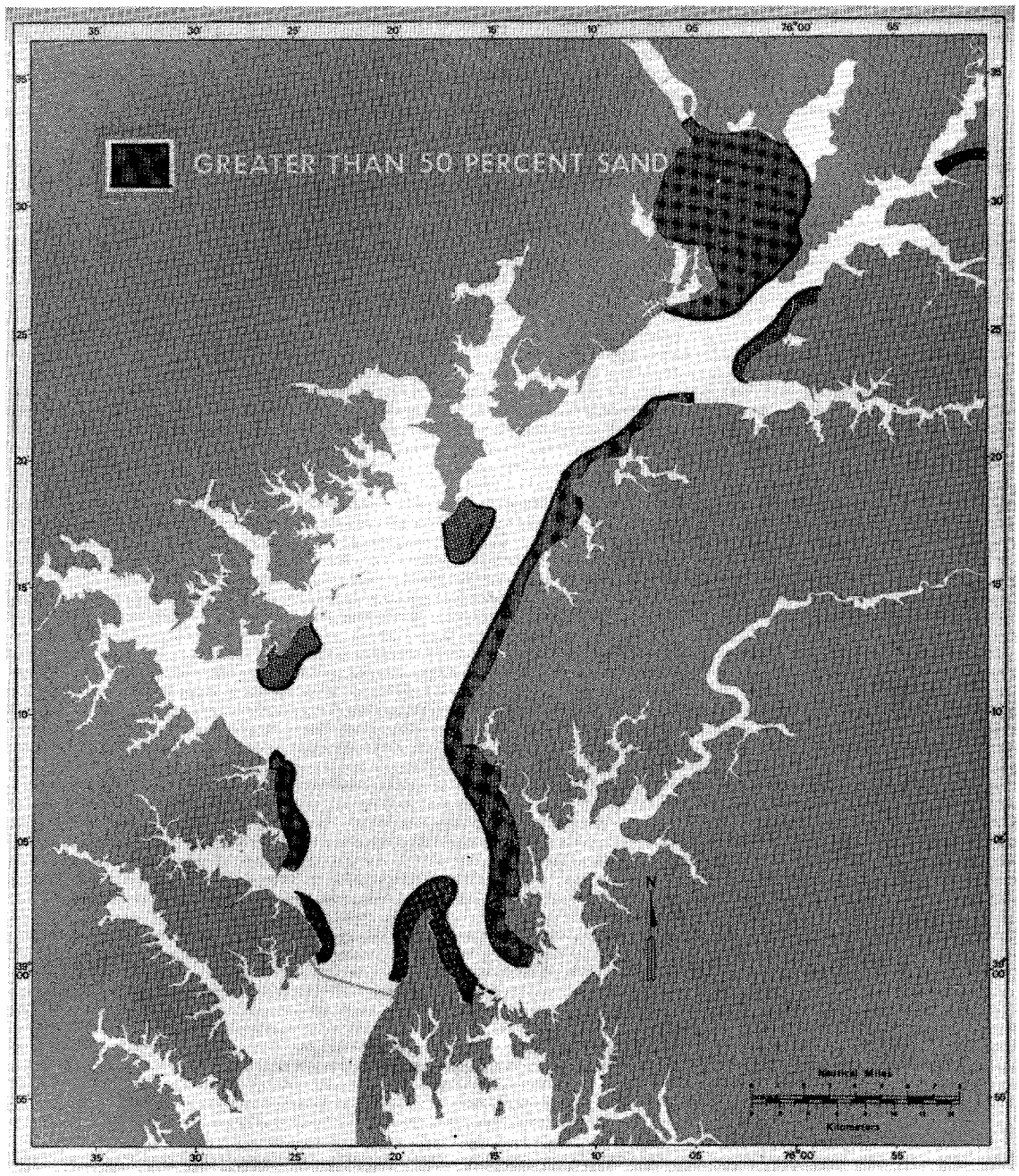

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Figure 4-4. Distribution of Sand in the Upper Chesapeake Bay. (Generally sand is confied to shoals along the Eastern Shore. Areas where sand is the dominant sedimentary component are shaded.)

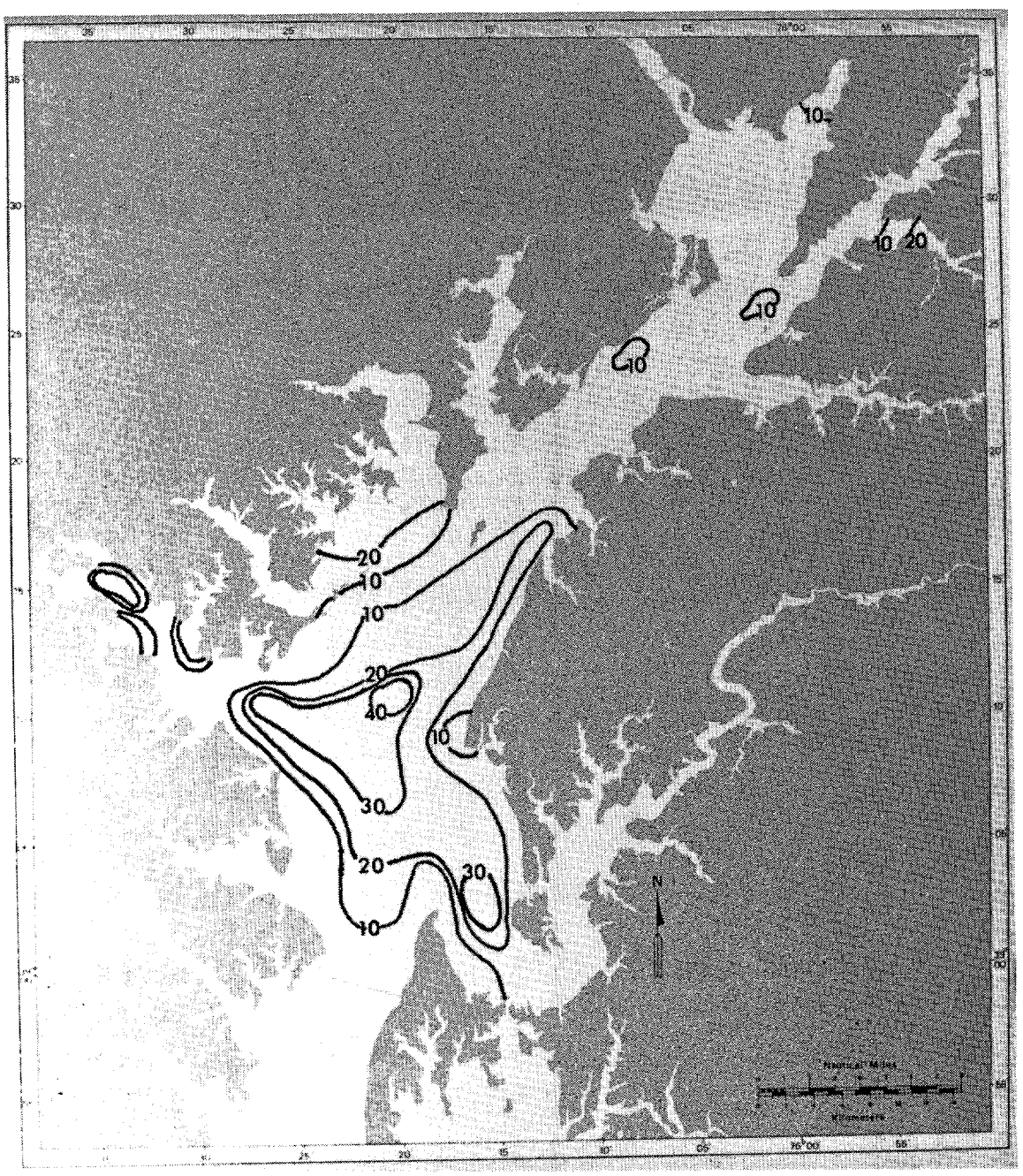

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Figure 4-5. The Silt-Sand Ratios in the Upper Chesapeake Bay. (Ratios indicate the prevalence of finer materials in the central part of the upper bay. Contours show distribution of ratio values.)

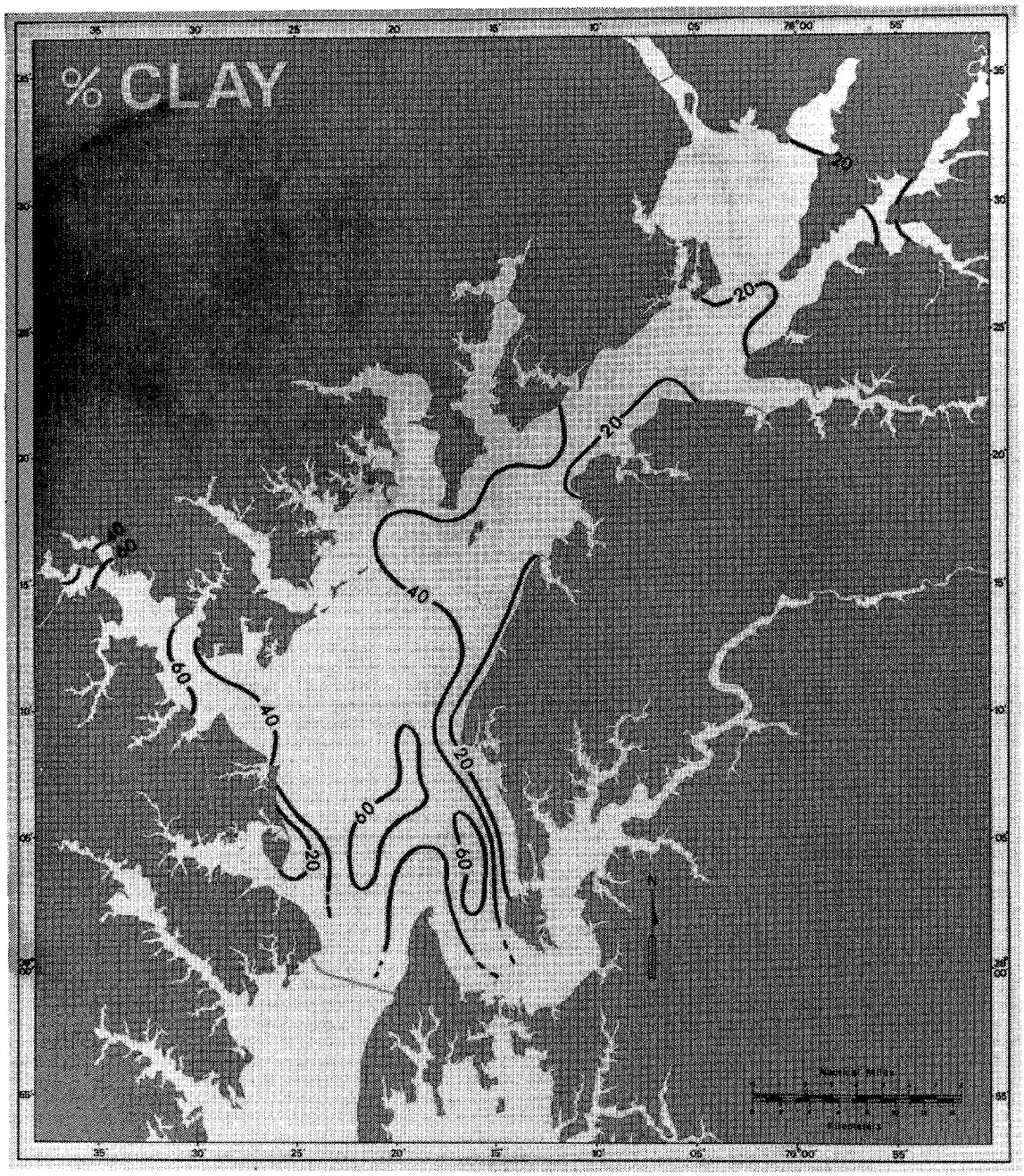

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Figure 4-6. Percent Clay in Bottom Sediments.
(Percentages are shown by contours. Highest concentrations are located in the central bay area where turbulent energy is minimal.)

From the earliest work reported by Ryan (1953), it has been known that the bulk of Chesapeake Bay sediment is fine-grained materials which settled out in the sluggish flow associated with the low gradients provided by the nearly flat coastal plain. Because of the low gradients of the mouths of tributaries, themselves estuaries to the larger Chesapeake Bay estuary, much of the coarse load carried by the rivers and streams is deposited before it reaches the bay.

As pointed out by Schubel and Biggs (1969), there are three sources of sediment to the upper Chesapeake Bay: internal, marginal and external (Figure 4-7). Although their classification was applied to seston (suspended material), it is equally applicable to bottom sediments. Application of this concept is evident (but not specified) in sedimentological studies as early as that of Hunter (1914) and much later by Kofoed and Gorsline (1966). The effects of shoreline erosion (marginal sources) as a major source of coarse materials is discussed by Slaughter (1966, 1967), Schubel and others (1972), and Palmer (1972a, 1973, 1974).

Figure 4-4 reveals a general trend toward high concentrations of sand in the marginal areas of the upper bay, and although the limited number of samples renders this map far from a complete inventory of sandy locations, several trends appear to emerge. It will be noted that predominantly sandy areas are common to the eastern shoreline of the upper bay, while such extensive shoals are lacking or reduced along the western margin. Consideration of the annual regional wind field indicates an overwhelming westerly component to all but late spring winds. Considering the relatively broad fetch and exposed nature of the eastern shore, it seems logical to assume a much greater rate of sediment generation through destruction of marginal deposits along the eastern margin of the upper bay than along the western shoreline. The major exception to marginal sources is that of the Susquehanna Flats, the large delta formed at the head of the bay, where much of the flood deposits from spring freshets accumulate. (This was the site of major accumulations of coarse materials following the record discharges accompanying Tropical Storm Agnes in June 1973). Due to a fortuitous break in the often cloudy spring skies, the peak flow for the 1974 freshet was photographically recorded by an ERTS-1 (Earth Resources Technology Satellite) orbit on April 3, 1974 (Figure 4-8.) In the course of studies under a separate contract, data for suspended sediment concentrations were obtained during this event. Size analyses showed that as much as 50 percent of the suspended sediment in the water column at this site was of silt size (62 to 4 microns diameter) and that concentrations of seston reached 45 mg/liter. Both parameters are higher than average; these freshet events must be considered significant occurrences with regard to total sediment budgets in the upper bay (Schubel, 1972).

An examination of the silt content in sediments of the upper Chesapeake Bay provides a significant insight into the hydraulic processes which affect the distribution of detrital materials in regional bottom sediments. These are the particles derived from pre-existing materials (rocks, sedimentary deposits, etc.) as opposed to particles resulting from biologic activity, agglomeration of fine particles into a single larger composite particle, and man's activity (slag, coal from mining operations, etc.).

Clay-size materials (particles having diameters less than four microns) are frequently involved in biologic and chemical processes which form agglomerated masses having settling velocities much higher than those for their individual constituent particles. Therefore, the evaluation of local hydraulic regimes based upon size analyses is biased to an unknown degree, depending upon the efficiency of agglomerative processes and the ensuing disruption of these agglomerates during processing of sediment samples for size analyses. In an effort to determine the local hydraulic processes affecting sediment distribution in the upper Chesapeake Bay, attention was directed to the finest particles which do not participate in agglomerative processes. These are the silts. The distribution of this size and composition was accomplished by scanning electron microscopy (SEM) and x-ray fluoresence. Representative SEM images, and distribution maps for selected mineral components, are shown in Figures 4-9 through 4-12.

Quartz is by far the most common mineral in the silt and the sand fractions of the upper bay's bottom sediments (Figure 4-10). Only one local area contains less than 80 percent quartz, and regional values are generally greater than 90 percent (Figure 4-9). The quartz silt commonly appears in two shapes: (1) as a rounded, generally equidimensional grain with surface textures displaying evidence of fluviatile abrasion (Figure 4-9b and c; see Krinsley and Margolis, 1969 for discussion of surface textures), and (2) as irregular plate-like chips with surface textures attributable to glacial grindings (Figure 4-9a). Muscovite (Figure 4-11) is the dominant mica in the bay, and in local areas can account for

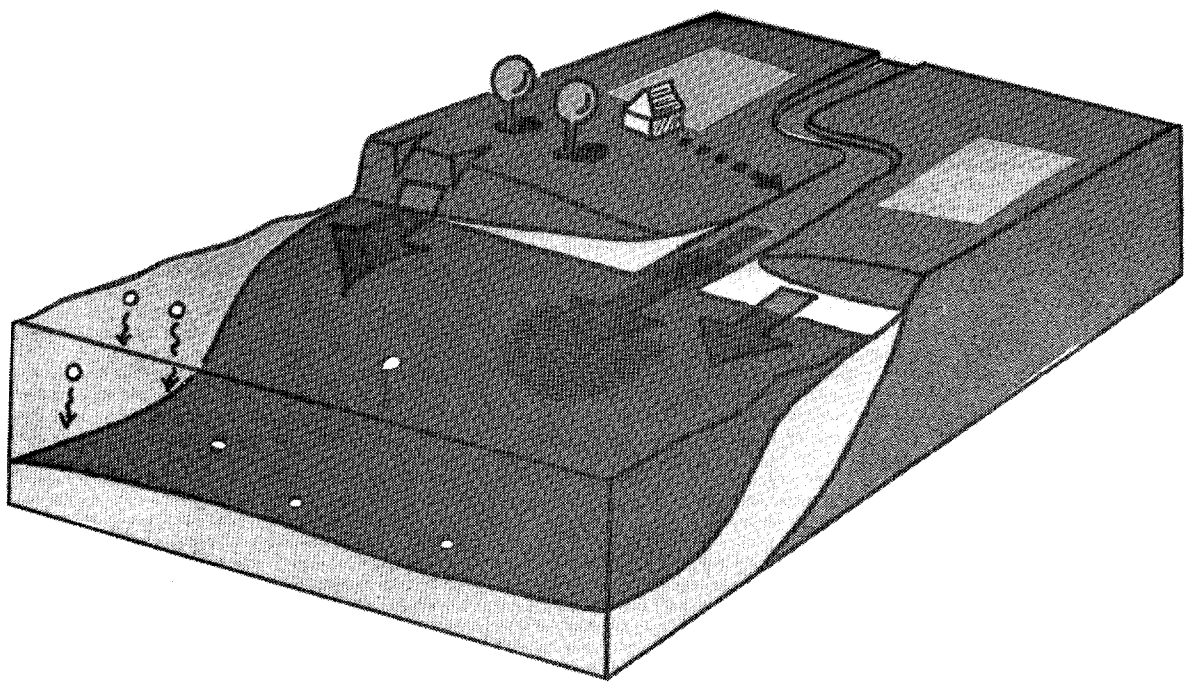

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Figure 4-7. The Three Sources of Sediment to the Bay. (Sources are shown in schematic form. Internal sources are biogenic and from resuspension. Marginal sources are those of shoreline erosion; external sources are the contributions from tributaries.)

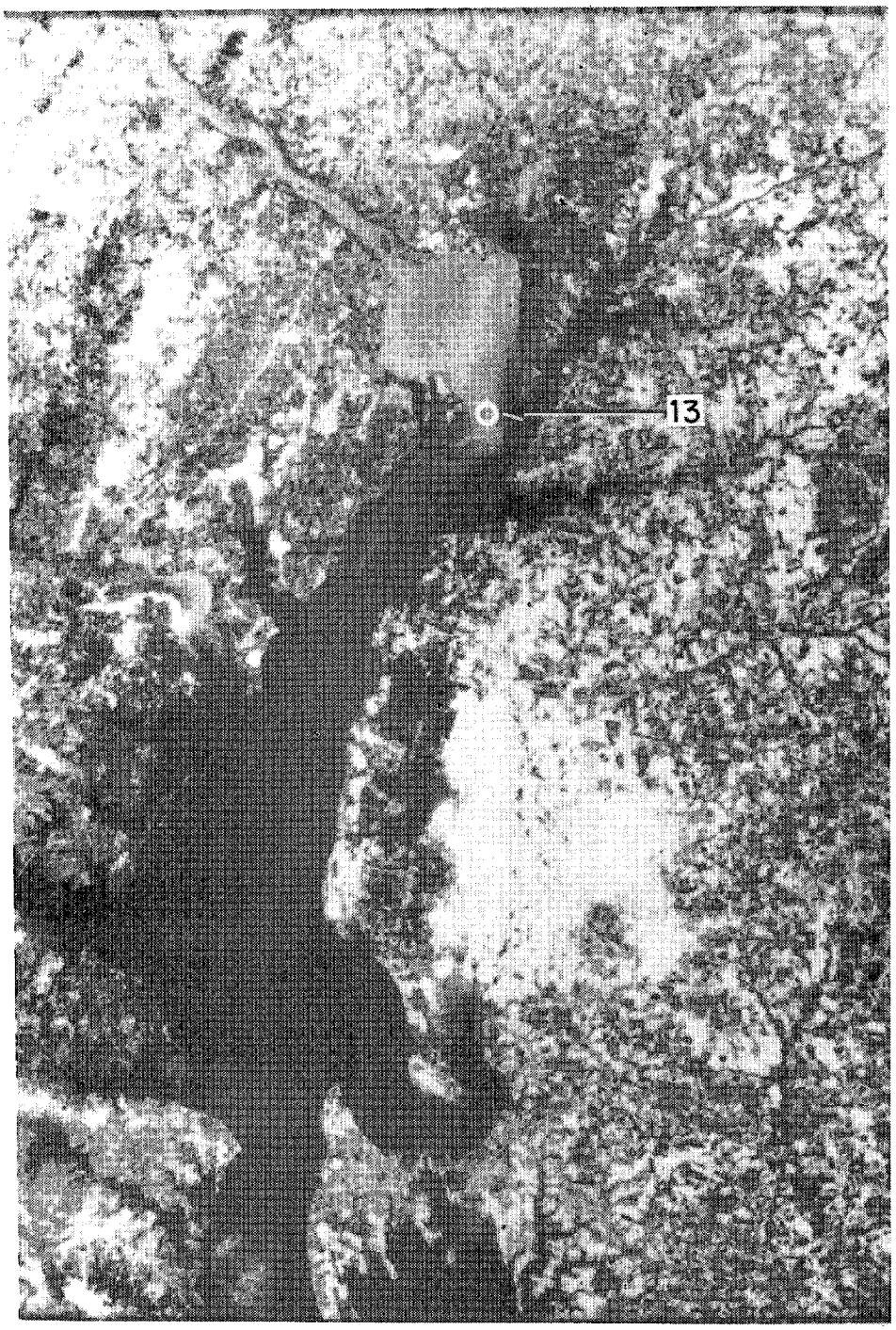

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Figure 4-8. Satellite (ERTS-1) Photograph of the Upper Chesapeake Bay, April 3, 1974. (Peak flows associated with the spring freshet have created turbid conditions at the mouth of the Susquehanna River. The station denoted by the figure 13 was occupied on this date.)

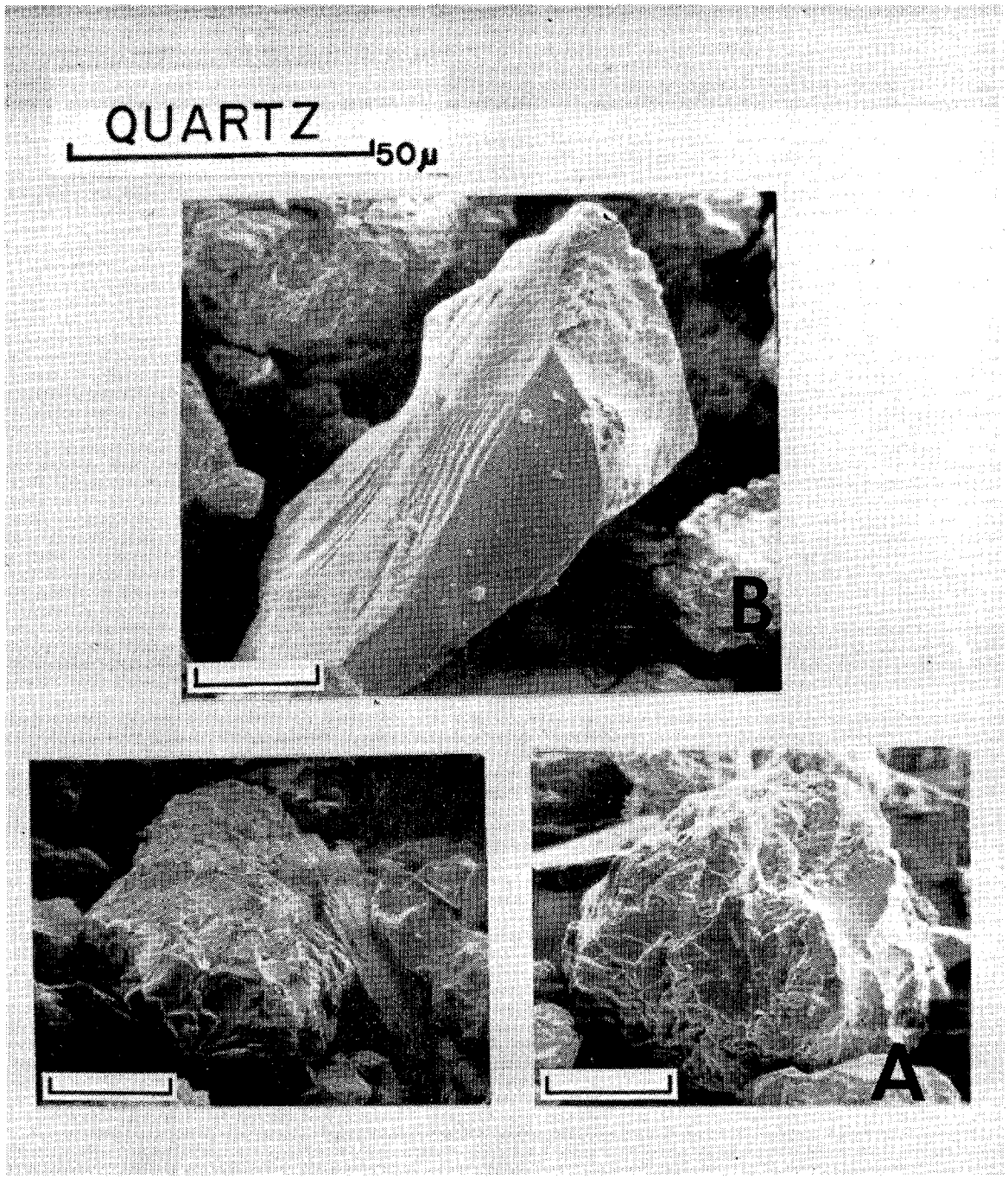

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Figure 4-9. Scanning Electron Microscope Images of Quartz Silt. (Bar scale is 20 microns in length.)

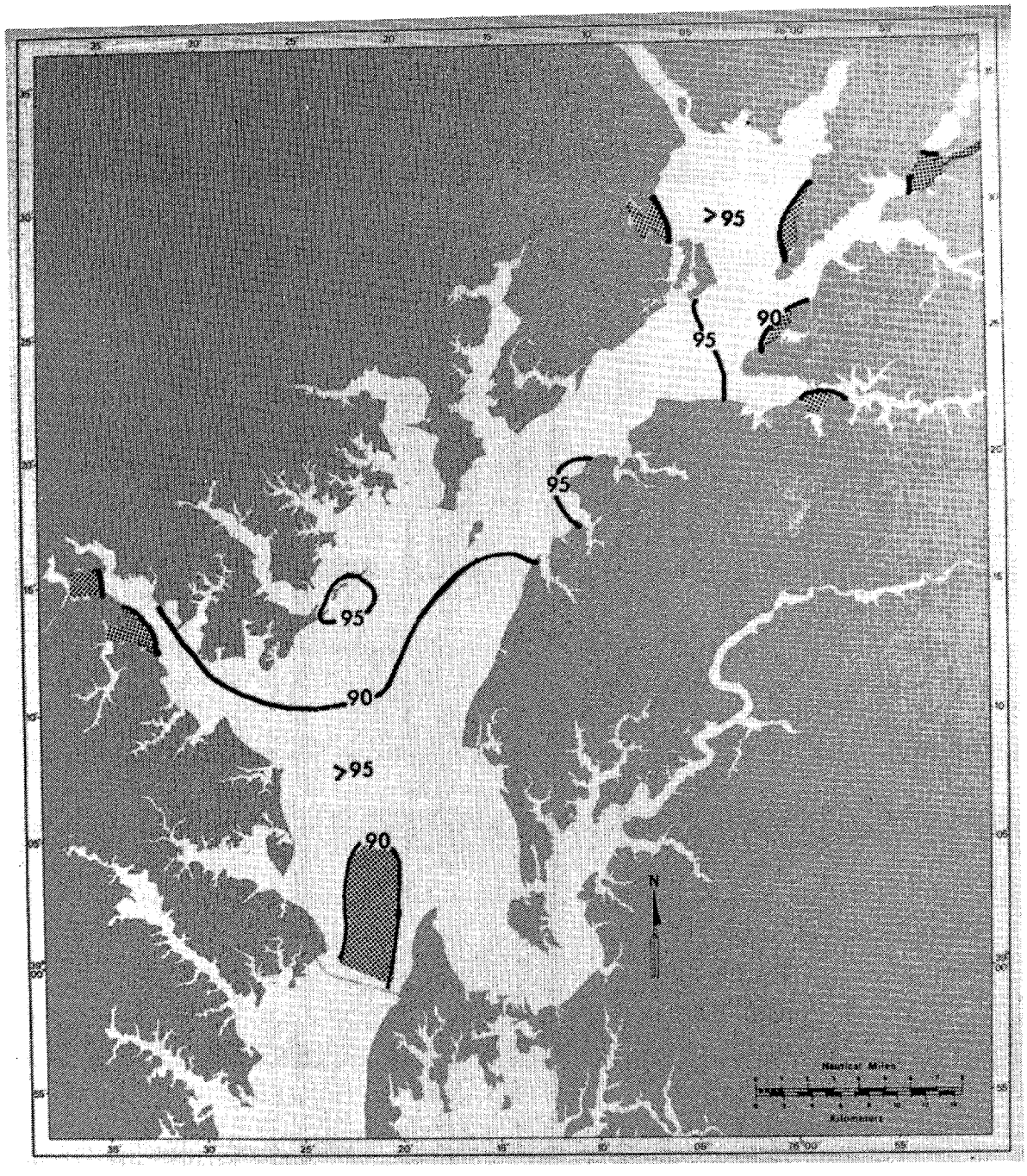

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Figure 4-10. Distribution of Quartz Silt in Bottom Sediments. (Contours are in percent quartz content.)

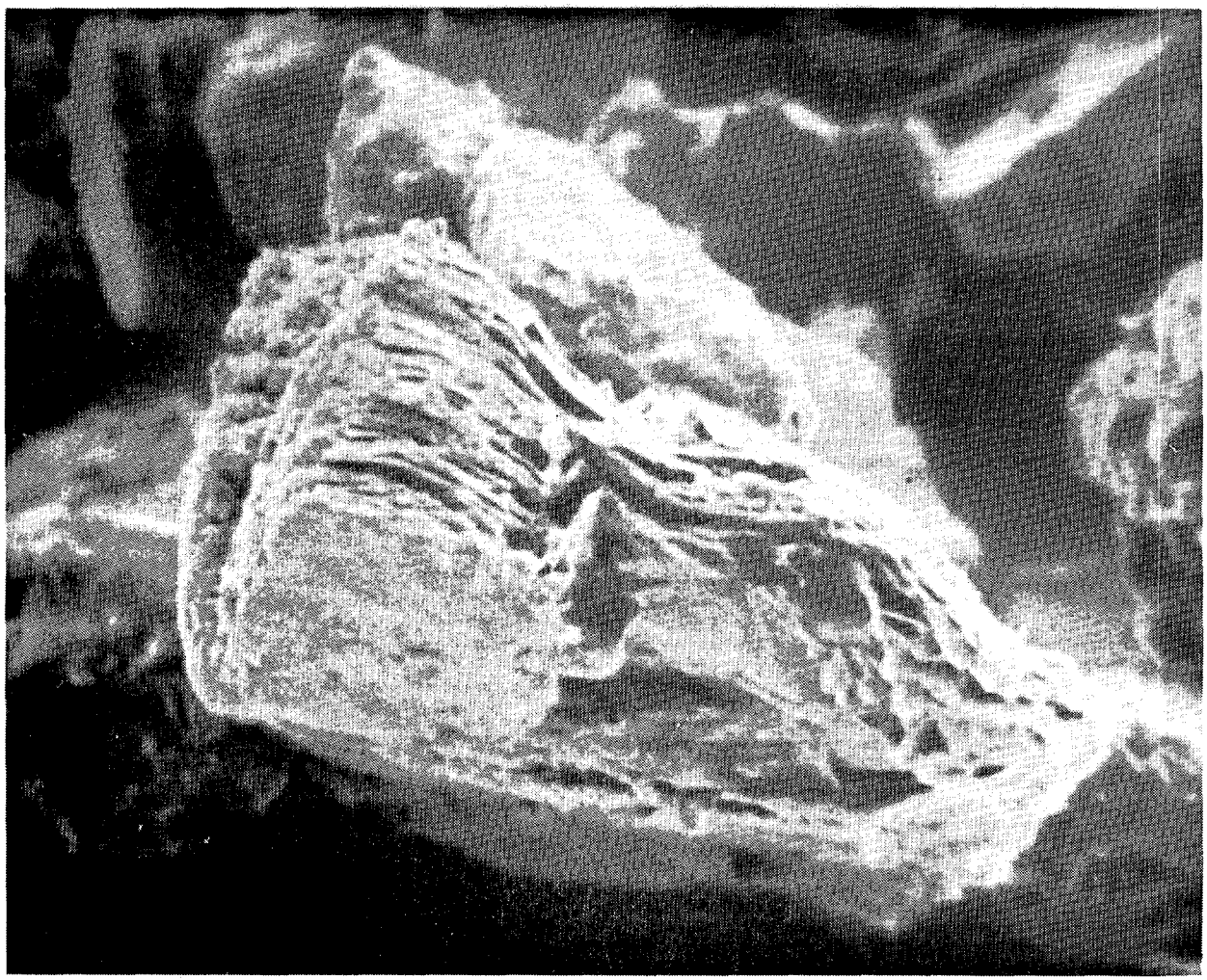

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Figure 4-11. Scanning Electron Microscope Image of Mica (Muscovite) from Bay Sediments.

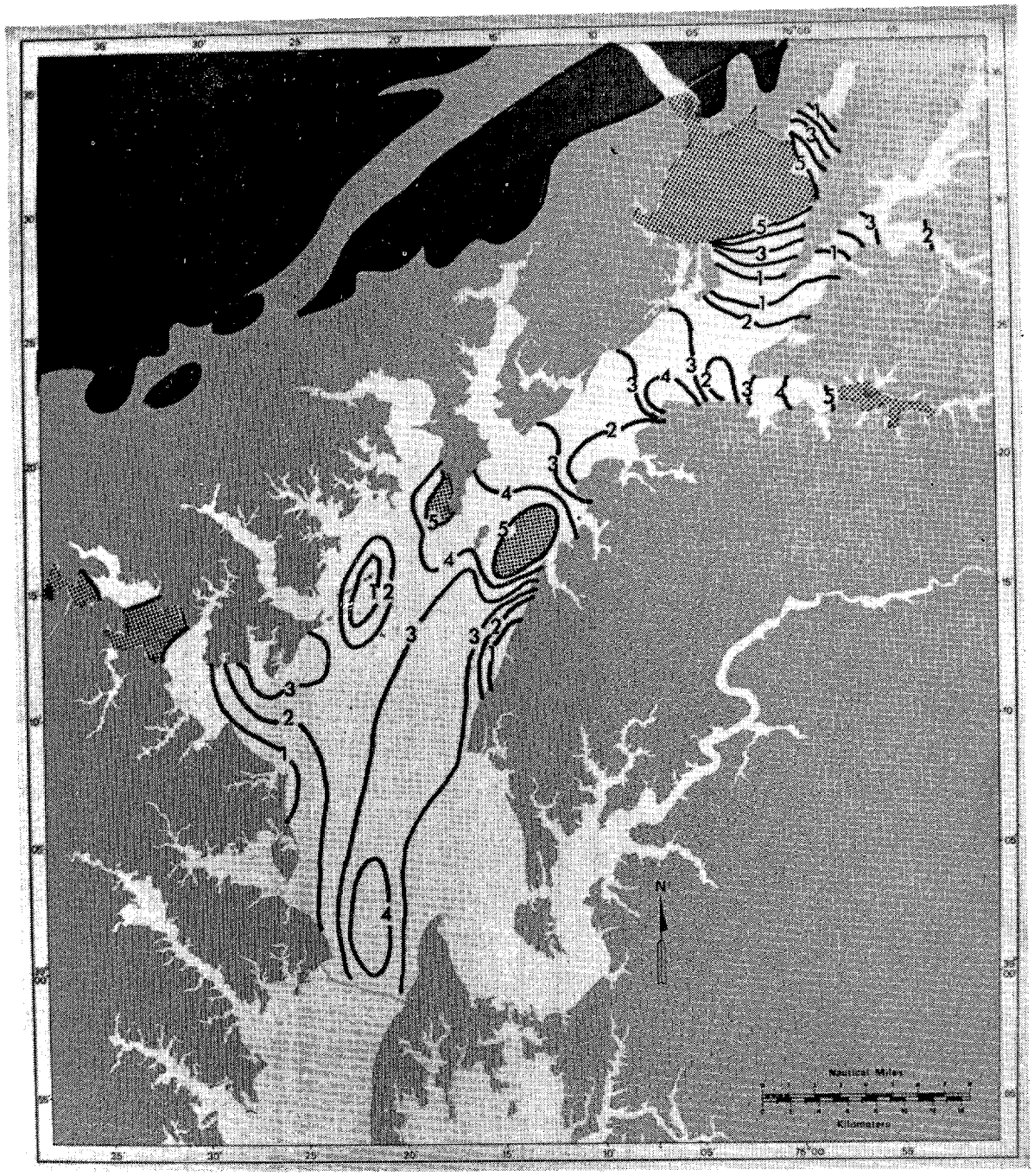

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Figure 4-12. Distribution of Mica in Bay Sediments.
(Contours are in percent.)

more than five percent of the total sediment (Figure 4-12). Glauconite (Figure 4-13) is a common accessory mineral throughout the upper Chesapeake Bay (Figure 4-14), and silt size particles display an etched appearance common to chemical weathering processes affecting grain surfaces. Slag and coal (Figure 4-15) are found in localized areas. The coal generally is concentrated in the headward portion of the upper bay, while the slag is centralized about the mouth of the Patapsco River (Figure 4-16). Biogenic components (Figure 4-17) are ubiquitous, reflecting internal contributions to sediments throughout the entire bay.

Analyses for clay mineralogy were not performed during this study, but the preliminary work of Palmer (1972a) in the Chester River and more detailed studies in the upper Chesapeake Bay by Owens and others (1974) reveal the following relative abundances: illite, 39.9 percent; kaolinite, 261 percent; montmorillonite, 21.1 percent, and chlorite, 12.9 percent. The general distribution of clays in the upper bay was shown in Figure 4-6.

4.1.4 Discussion

The centralization of clays and silts in the broad area between the Patapsco and Chester Rivers is a reflection of the regional hydraulic regime. Here the circulation is more sluggish than in the channel area which occupies the eastern margin of the upper bay. Within this area, sand-silt ratios reach a maximum value of 47 (see Figure 4-5), and clay content also is higher than elsewhere in the upper bay (see Figure 4-6). Consideration of published circulation patterns in this section of the bay (ESSA, 1968) and discussions with workers participating in this study imply that, although a true gyre does not exist in this broad segment, tidal currents are indeed slower than the average velocities encountered in more restricted cross-sections of the upper Chesapeake Bay. For this reason, one may consider this region to be a sediment sink in that diminished current speeds promote the settlement of finer grained materials. Consideration of the sand-silt-clay ratios for samples with respect to particle size provides some insight into mechanism which lead to the deposition of the finer fractions of the bay's sediment load.

Sediment size data may be conveniently presented on a triangular diagram in which the apices represent the three major size components (Figure 4-18). If the sediment recovered from the floor of the bay moves as bedload, that is, in traction along the bottom in response to fluid drag, then there should be a regular diminution of size (usually quantified as a mean or median particle diameter for a given sample) in a downstream direction (Figure 4-19, Left). This is the concept of the graded river in which the materials deposited in the river bed are adjusted to the flow regime in the river at that point. The coarser particles are left upstream in the region of swift flow, while the finer materials are carried past the site to be deposited in an area of more tranquil flow. Under these conditions where bedload dominates, the distribution of a suite of samples would appear as shown in (Figure 4-19, Left). But as many workers have shown, the silt and clay fractions of sedimentary deposits have particle diameters such that the threshold velocity required to initiate movement (to pick up a particle) is much greater than that velocity required to maintain it in suspension (Smith and Hopkins, 1972). For this reason, one can expect that silt and clay, once entrained in the fluid, cannot travel as bedload but only as a suspended load (part of the seston discussed in this section). Under such circumstances, one may expect a distribution of sample points similar to that shown in Figure 4-19, Right. In this figure, the distribution of points representing samples is scattered across the central part of the diagram, and the poor sorting implies a fluctuation in the transport energy which in turn determines the nature of the deposit. This figure displays the normal distributional pattern of sample points from estuarine studies (Palmer, 1972; Faas, 1972; Nichols, 1972; Merryman and Palmer, 1975), and it holds true for upper Chesapeake Bay samples taken for this study (Figure 4-20).

If one compares the relatively high threshold velocity required to entrain a silt particle with the relatively low settling velocity for the same particle, it is apparent that there will be poorer sorting with a decrease in mean particle size. This is because deposition will take place only when turbulence, and not necessarily velocity, diminishes to a value below that of settling for a given grain size. With decreasing particle diameter, the point is approached where sustained background turbulence exceeds settling velocity, and the particles may be considered to be in permanent suspension. According to Schubel (1971), upper Chesapeake Bay turbulence may be considered to be about 10^{-3} cm/sec, which corresponds to a settling velocity for particles having a diameter of four microns (at 20°C).

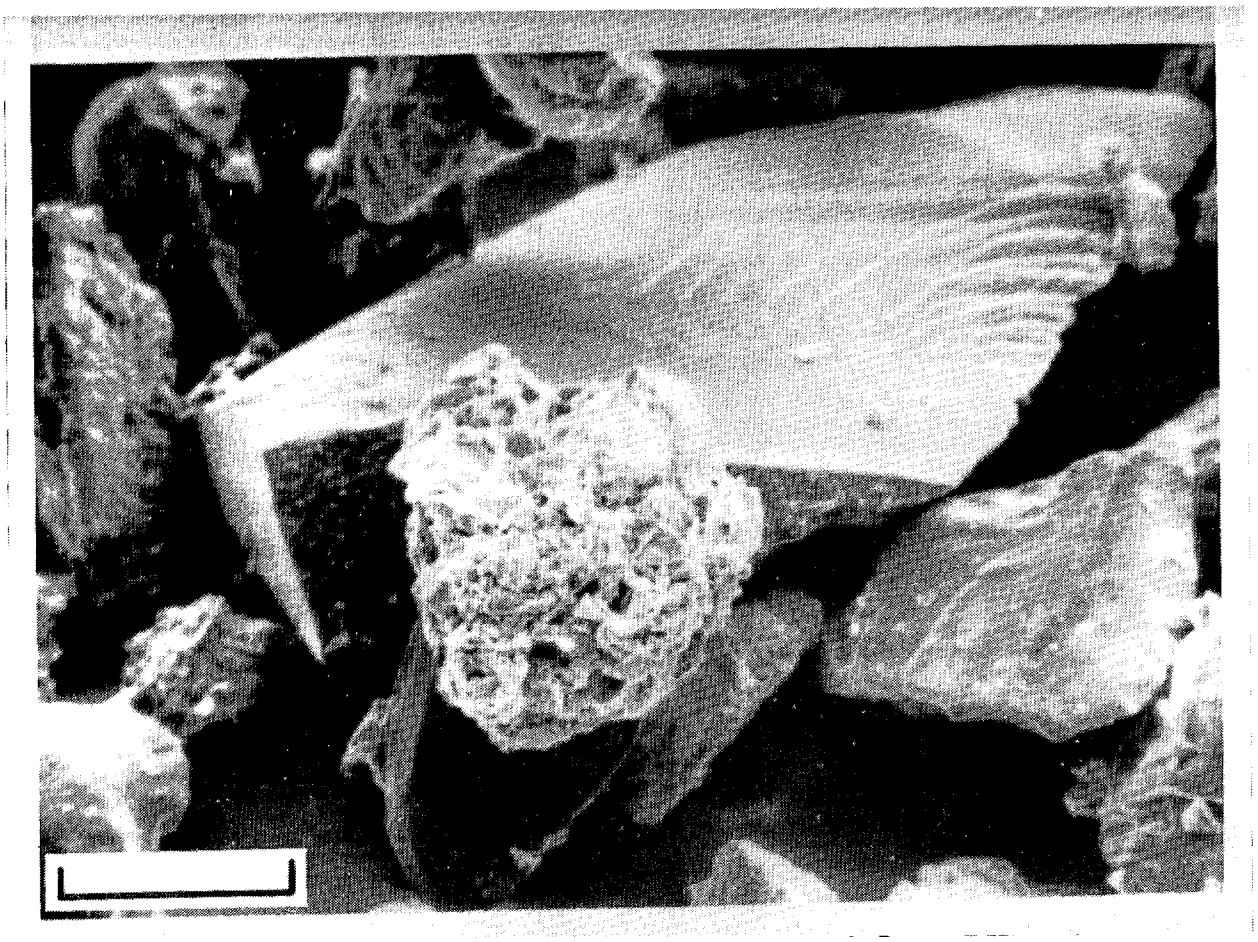

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Figure 4-13. Scanning Electron Microscope Image of Glauconite Grain. (The grain is front center of angular quartz silt particle. Scale bar is 20 microns.)

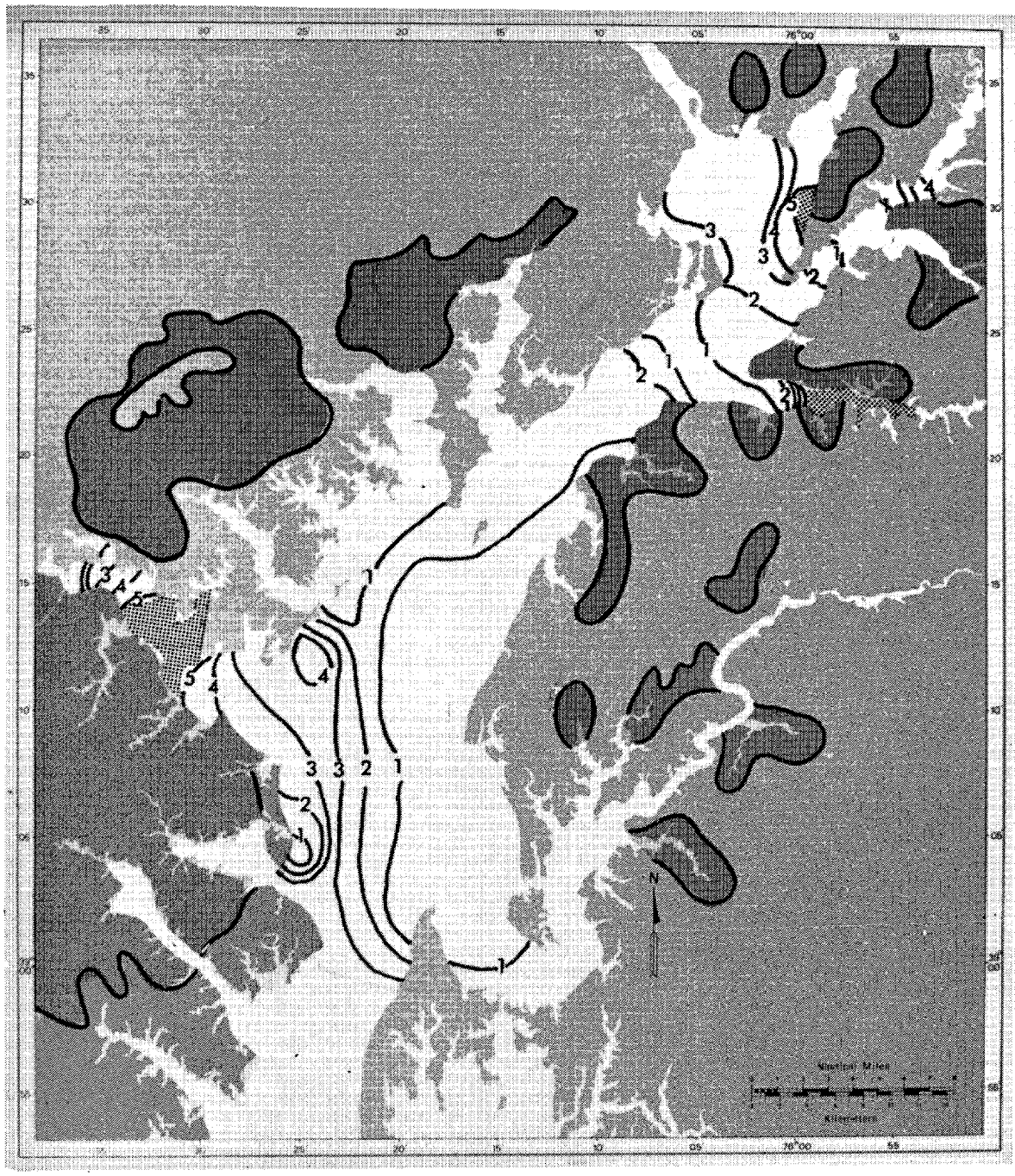

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Figure 4-14. Distribution of Glauconite in Bay Sediments. (Contours are in percent. Broad grey arrows are slag trajectories from the Patapsco River; narrow black arrows are coal trajectories from the Susquehanna River.)

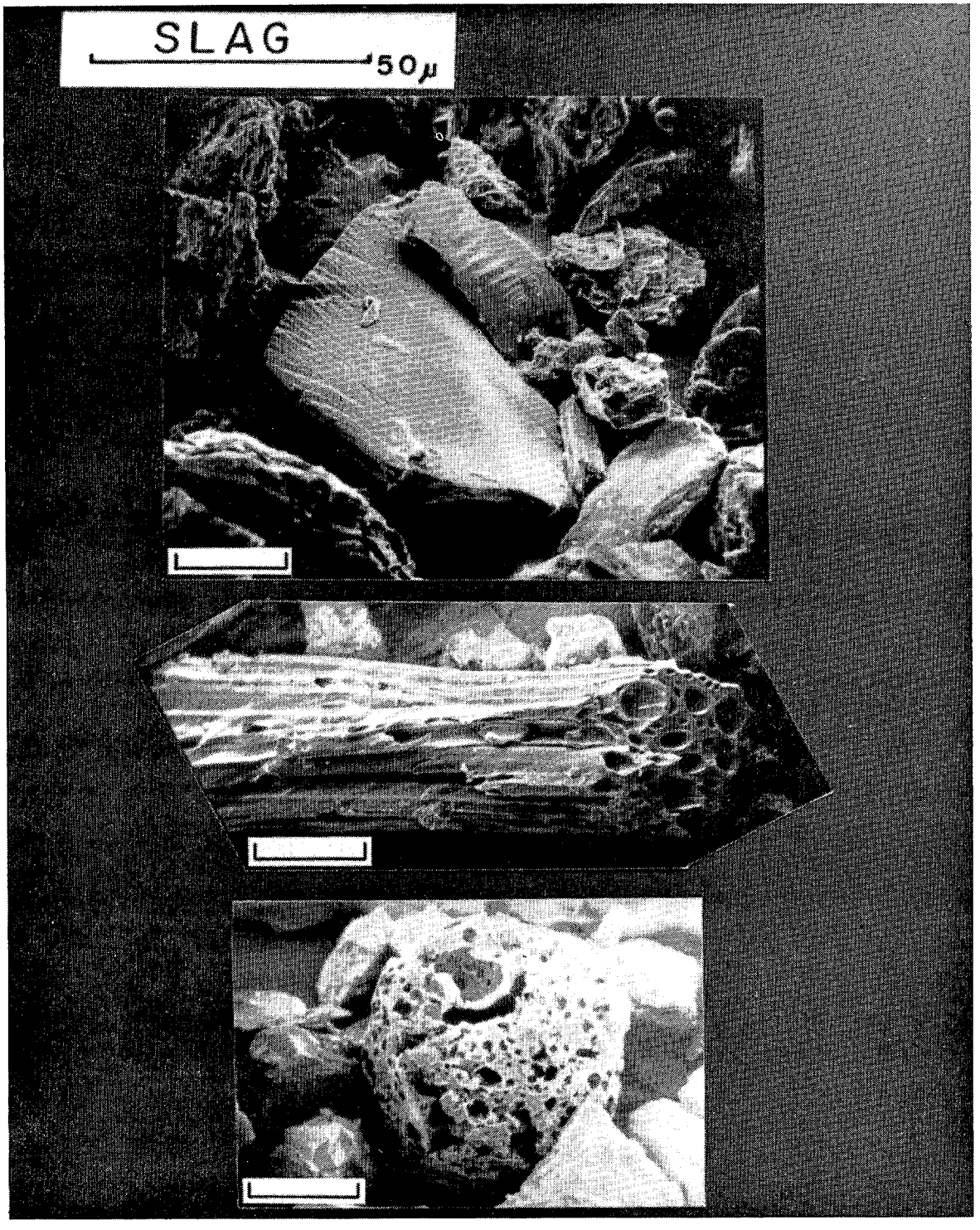

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Figure 4-15. Scanning Electron Microscope Images of Slag Fragments in Upper Bay Bottom Sediments. (Scale bar is 20 microns.)

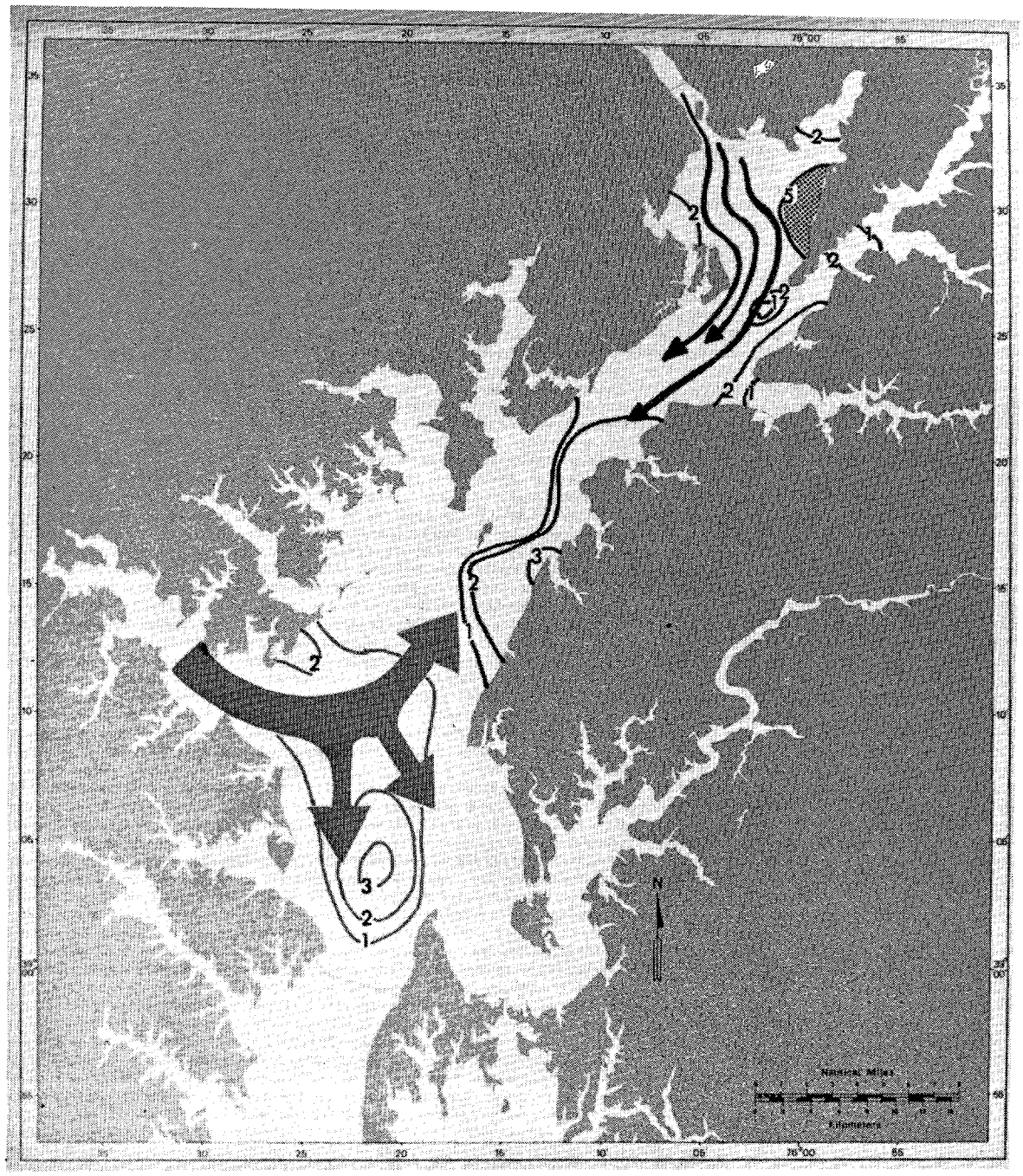

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Figure 4-16. Distribution of Slag and Coal in Bottom Sediments. (Contours are in percent.)

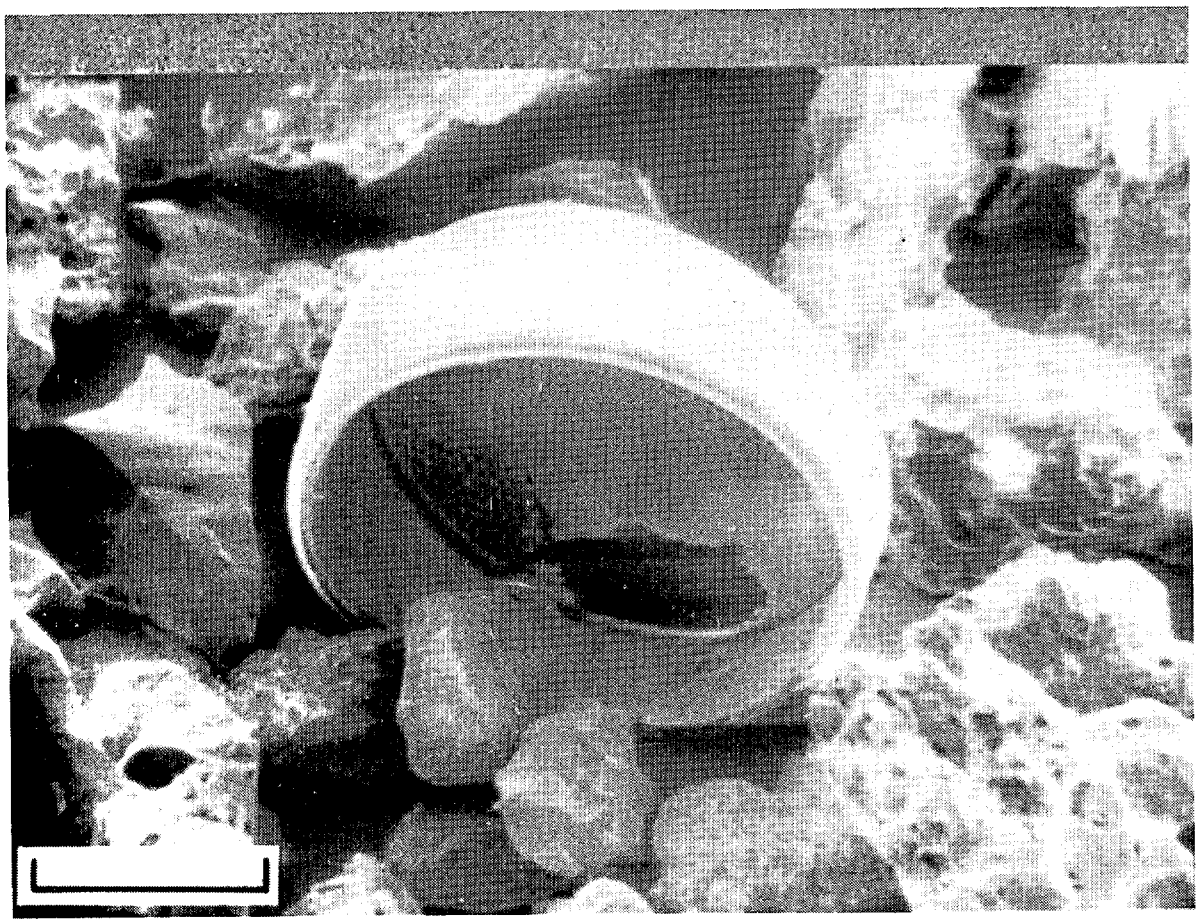

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Figure 4-17. Diatom Frustule Comprising Some of the Biogenic Debris in Bottom Sediments of the Upper Bay. (Scale bar is 20 microns.)

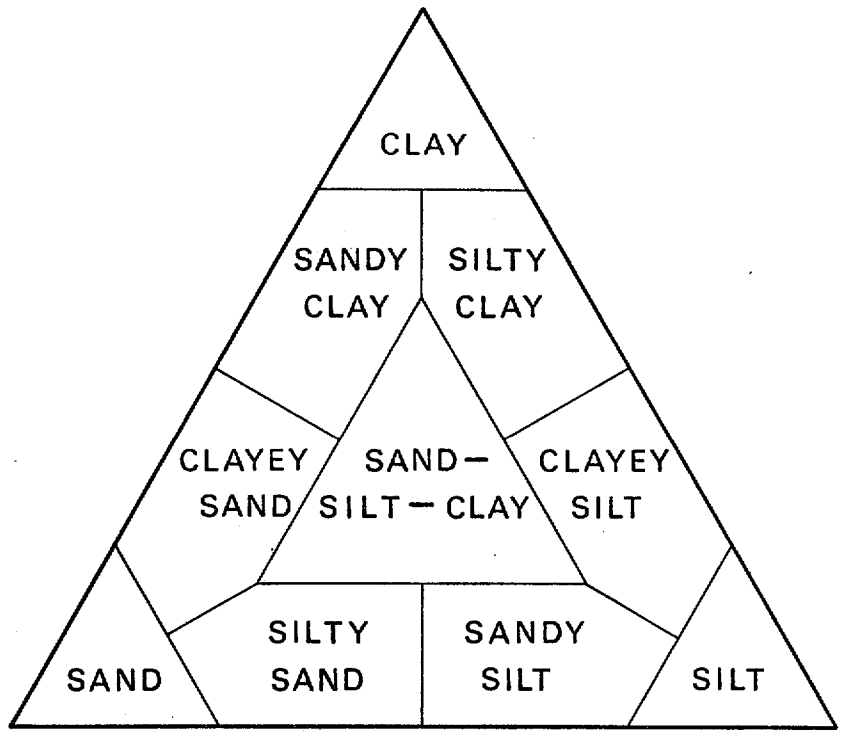

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Figure 4-18. Triangle Diagram for Displaying Sediment Size Characteristics.

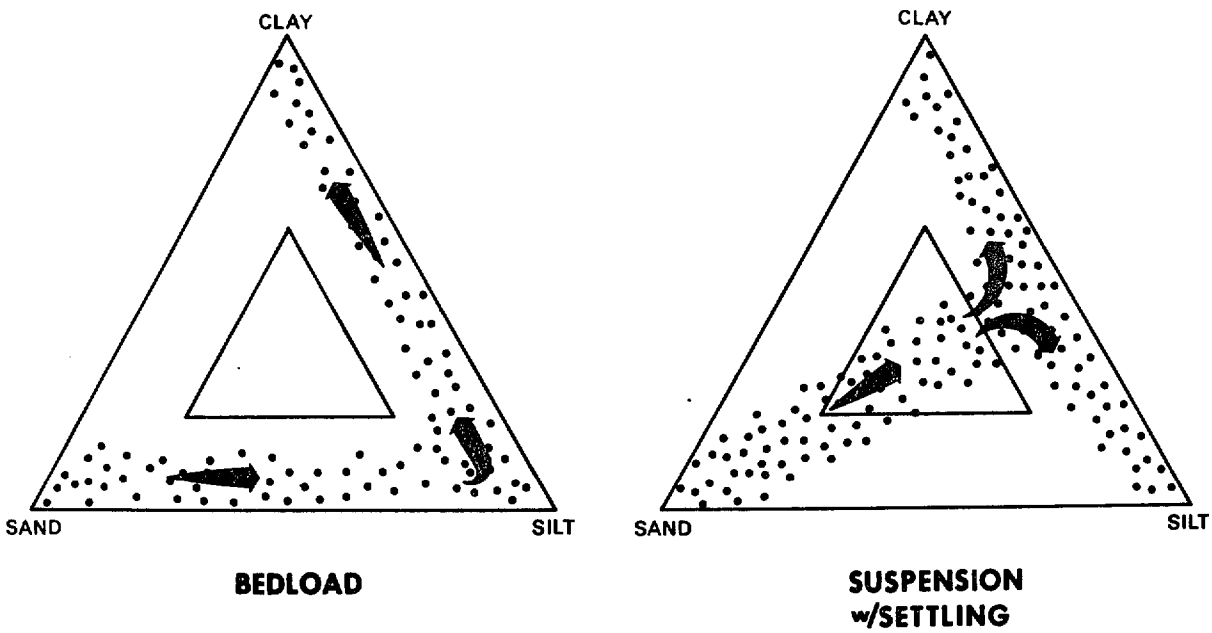

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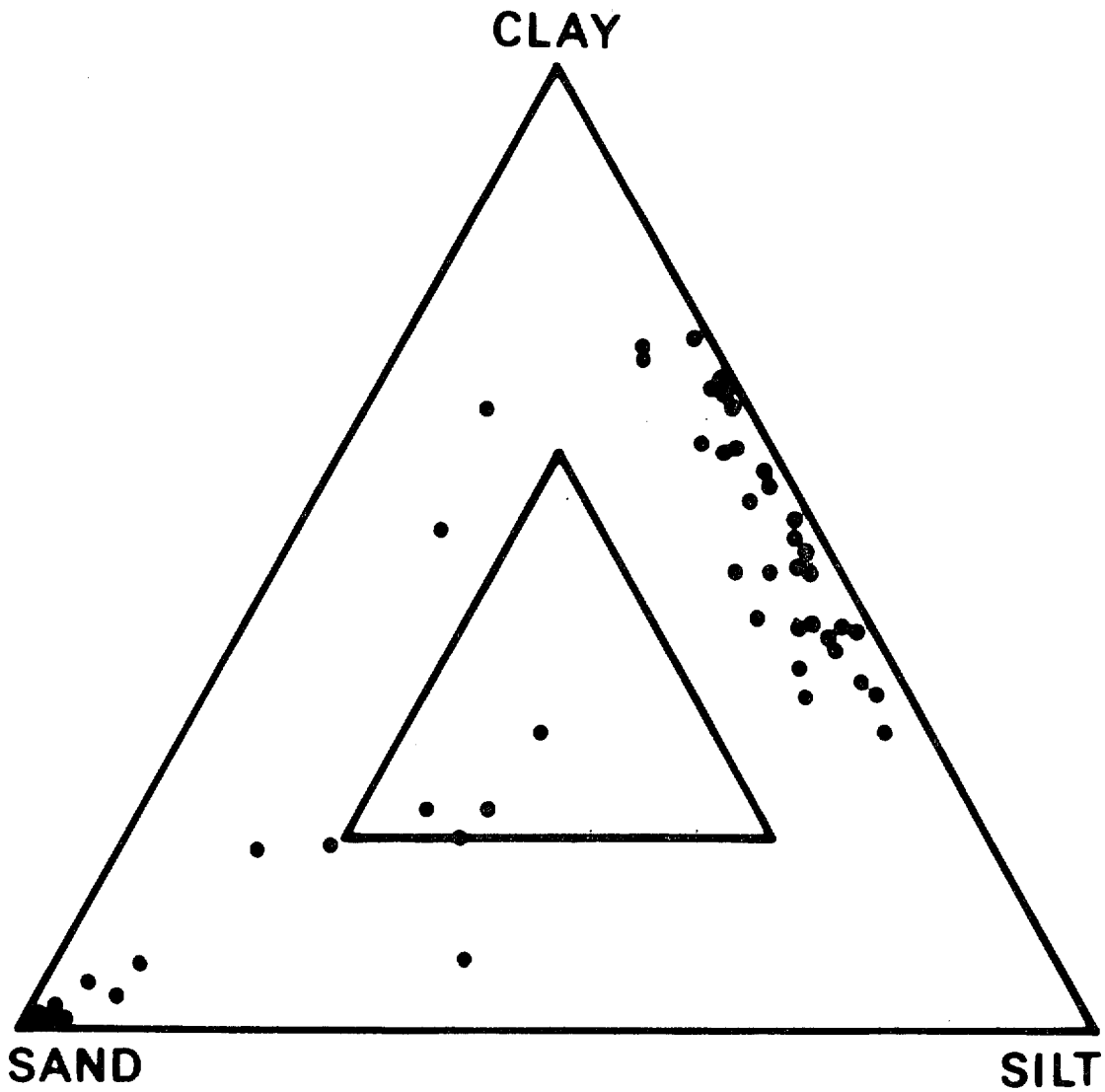
Figure 4-19. (Left) Pattern of Sample Distribution for Bedload Materials. (Trend for graded deposits is shown by arrows; with diminishing currents the deposit decreases in size from sand, through silt, and ultimately to clay.)

(Right) Pattern of Sample Distribution for Deposits Resulting from Suspended Sediment. (Once placed in suspension, silt size particles are carried as suspended load and deposited with various mixtures of sand and clay.)



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Figue 4-20. Triangle Diagram for Bottom Samples Recovered During the Upper Bay Survey. (Note correspondence with Figure 4-19, Right.)

However, the bottom sediments contain finer material which have settled from suspension during periods of quiescence, and the bulk of suspended sediment (the modal class) lies in a size range of 0.60 to 0.74 microns (Figure 4-20). Inasmuch as turbulence is highly variable and transitory, local variations in size distribution with both space and time are common. Yet, it is possible to determine trends such as those shown by Figures 4-5 and 4-6 which reveal hydraulic controls on bottom sediment distribution. Thus, Figure 4-5 provided a picture of longterm integration of the turbulence and velocity fields within the upper bay, and it may be considered an indicator of the sinks for materials carried by the five fractions of sedimentary deposits.

4.1.5 Summary

The purpose of the bottom sampling program was to determine the geographic distribution of sediment types and to seek correlations between size distribution and concentrations of various chlorinated hydrocarbon compounds in the upper Chesapeake Bay. Sediment distribution has been covered in previous sections, but in summary, we may present the concentration data compared to size.

Figures 4-21 through 4-23 display concentration values for most of the bottom sediment samples described previously. In all three cases, there is a general upward slope as size diminishes and concentration increases. In Figure 4-21, the concentration increases. In Figure 4-21, the concentration of PCB (Aroclor®1254) averages about 0.09 ppm., with maximum values reaching well over 1 ppm. The same appear valid for the total PCB, but the average here is somewhat higher centered around 0.15 ppm (Figure 4-22). Total DDT values are lower (Figure 4-23), with an estimated average about 0.05 ppm, although this figure relies on fewer data points than the other two figures.

The implication drawn from these three plots is similar to that established for the Chester River Study (Clarke, et al., 1972) that an increase in concentration of both chlorinated hydrocarbons and trace metals (not addressed here) will be found in the finer fractions of natural sediments. Mechanisms for such situations are discussed in that report and will not be repeated here. However, one may make comparisons between the two regions, since analytical and sampling techniques were identical for both the Upper Bay Survey and the Chester River Study. Comparison of the total PCB concentration between the two areas indicates that in the upper Chesapeake Bay, bottom sediments generally carry a greater burden of PCB's than do those of the Chester River, a major eastern shore tributary to the upper bay. Figures 2 through 13 in Clarke et al. (1972) show a general relationship of spring and early summer data points which fall between 0.15 and 0.25 ppm. This is the general range for many points from the upper bay (Figure 4-22), but about one-third of the latter lie above 0.3 ppm. Such values were not recorded during the Chester River Survey.

Common values of total DDT for the Chester River fall between 0.01 and 0.05 ppm. (Figures 2 through 15 of Clarke et al., 1972). However, more than one-half the bottom samples from the upper bay showed values in excess of 0.05 ppm., the upper limit for Chester River materials. The conclusion which must be drawn from Figure 4-23, as well as from the PCB data shown in Figure 4-22 is that the upper Chesapeake Bay sediments in certain areas contain relatively high concentrations of these chlorinated hydrocarbon compounds. The higher values on these two figures are generally attributed to their proximity to, or recovery from, the Patapsco River. Other data presented elsewhere in this report display the concentrations and locations in graphic form (Chapter 6, Figures 6-2 and 6-3).

It seems clear that the concentration of fine-grained sediments in the central part of the upper bay, as shown in Figures 4-5 and 4-6, may form a sink for much of the chlorinated hydrocarbon compounds originating in the Patapsco River tributary. This appears to be the case with trace metal concentrations described by Helz (1974), and it is suspected that the same is true for PCB, DDT, and the other compounds of local origin. If this is the case, much of the material introduced by various local tributaries and from various industrial activities will be trapped in the region immediately outside (and also within) this tributary. For this reason, plans for maintenance and/or new dredging activities the area where these materials may be accumulating should be carefully studied prior to the dislocation and transport of burdened spoil materials. As Figures 4-21 through 4-23 show, there can be an order of magnitude difference in concentration for a given size class, suggesting that local variations may be quite high in relation to the total variance in concentration. Again, such distributional variations suggest that specific studies of local areas, as opposed to reconnaissance surveys, should be implemented for specific dredging projects involving the removal of materials carrying significant burdens of chlorinated hydrocarbons.

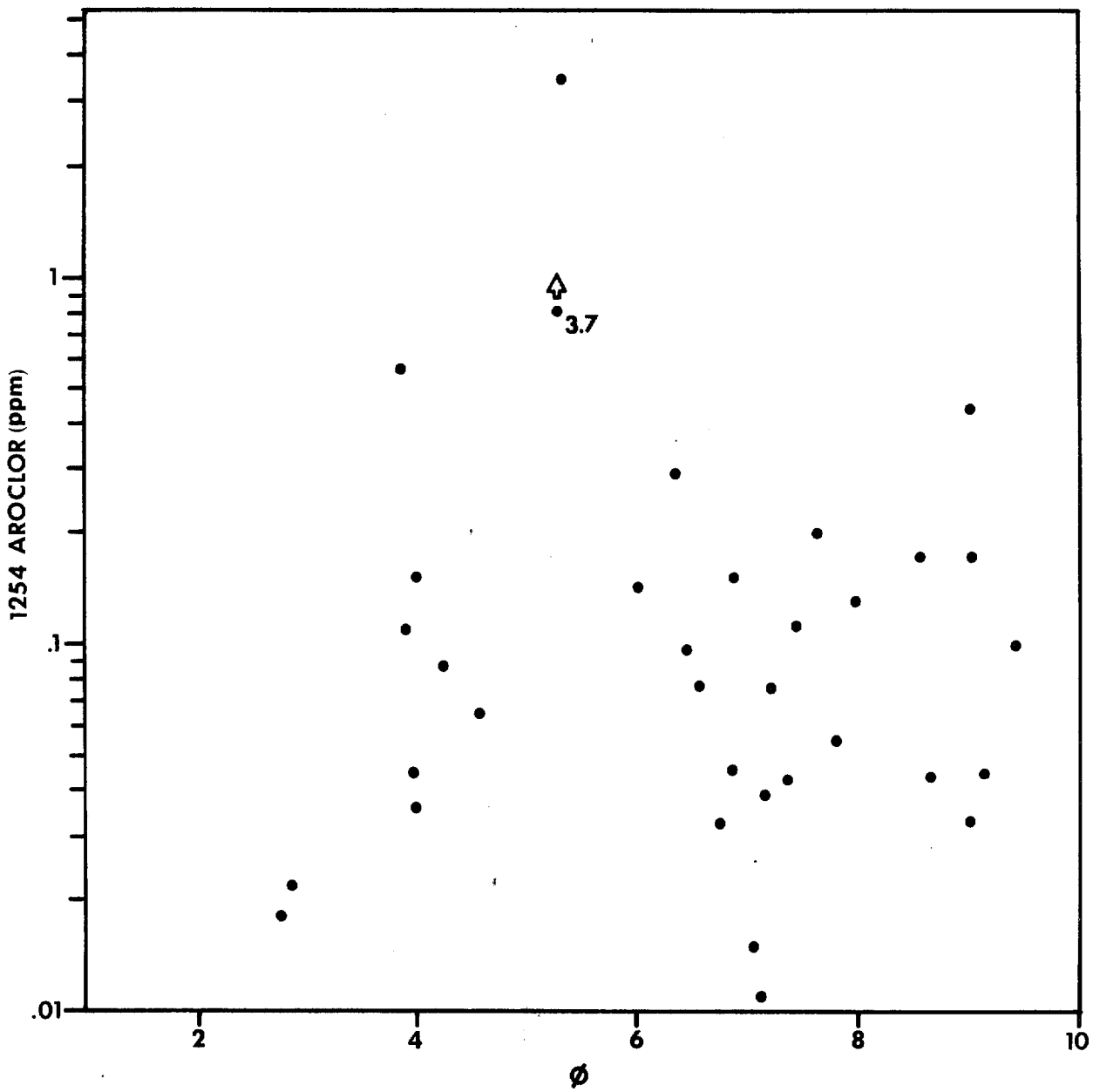

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Figure 4-21. Comparison of Mean Grain Size, in Phi Units.
(The concentration of Aroclor® (PCB-1254 is in parts per million.
One point lies at 3.7 ppm, off graph at top.
In phi notation, larger values compare to smaller particle size.)

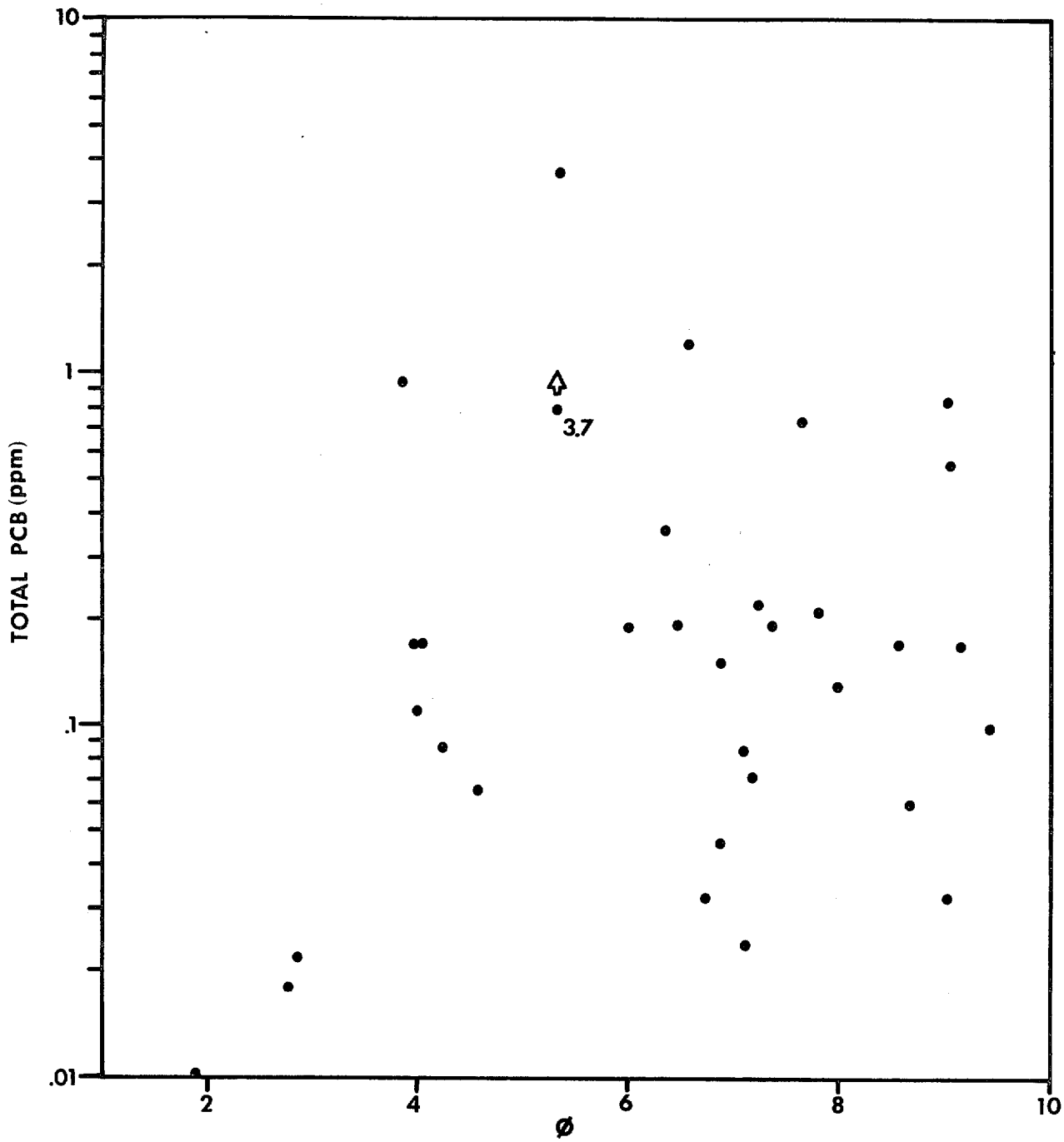

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Figure 4-22. Comparison of Mean Grain Size, in Phi Units.
(The concentration of total PCB, is in parts per million. One point lies at 3.7 ppm off the graph at top. In phi notation, larger values compare to smaller particle size.)

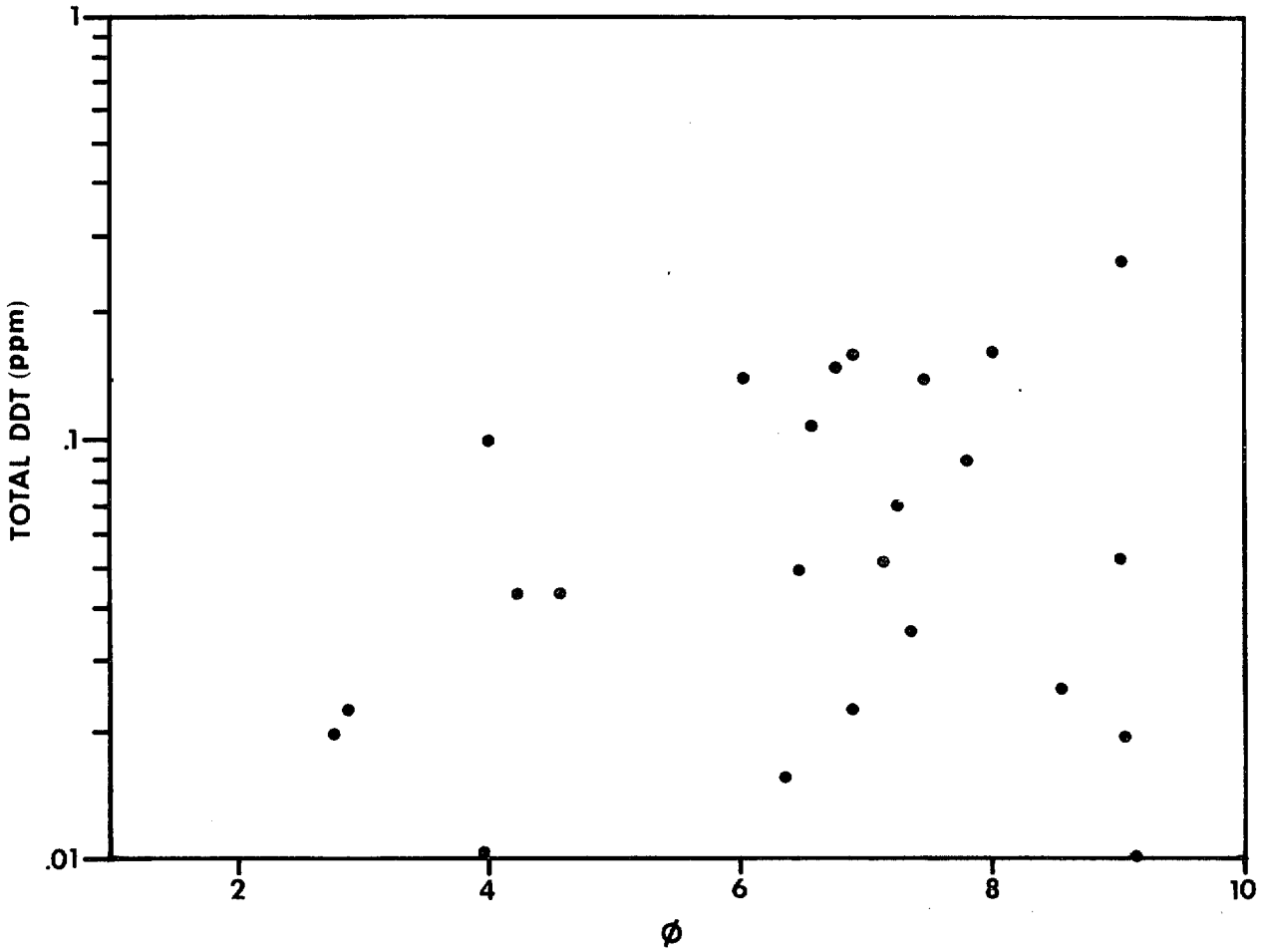

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Figure 4-23. Comparison of Mean Grain Size, in Phi Notation. (The concentration of total DDT is in parts per million. In phi notation, larger values correspond to smaller particle size.)

To confirm the premise that finer grain sizes are enriched in chlorinated hydrocarbons, an analysis was performed on different size fractions of two bulk samples one from the Baltimore harbor area and one from the mouth of the Chester River. The samples, consisting of about 18 kg (40 pounds) of sediment, were placed in a large container with distilled water, stirred, and left to settle. At appropriate intervals, a tray was placed in the suspension and left in place to collect fine-grained sediment as it settled through the water. Then, film of sediment was removed, classified for grain size, and analyzed for chlorinated hydrocarbon content. The results of this experiment are presented in Figures 4-24 through 4-26.

The concentration of chlorinated hydrocarbons as a function of grain size is clearly evident from these tests. With a decrease in grain size of only two phi units, a pronounced increase in concentration was evident. In addition, with but one exception, the Chester River samples were significantly lower than similar sized materials from the Baltimore harbor. In view of the data presented elsewhere in this report, this was not surprising, and this simple test confirms the fact that the finer fractions of the upper Chesapeake Bay sediments are carrying the major part of chlorinated hydrocarbon residue in the aquatic environment.

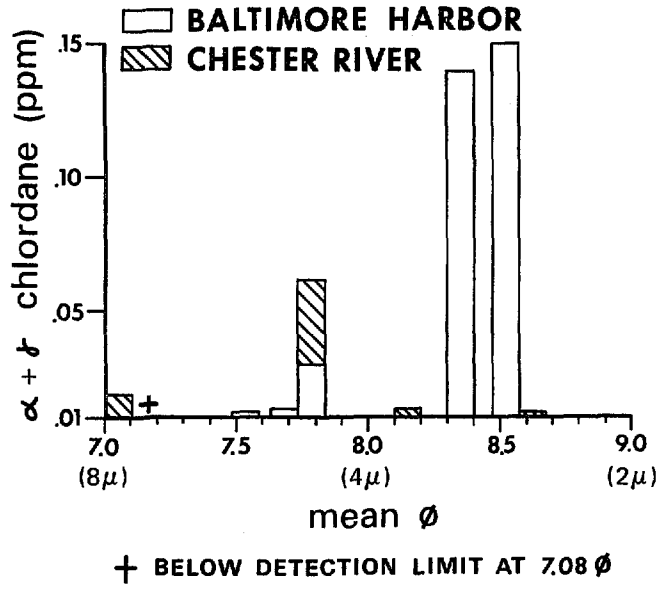

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Figure 4-24. Chlordane Concentrations in Bottom Sediments from Baltimore Harbor and the Chester River (depicted as a function of grain size.)

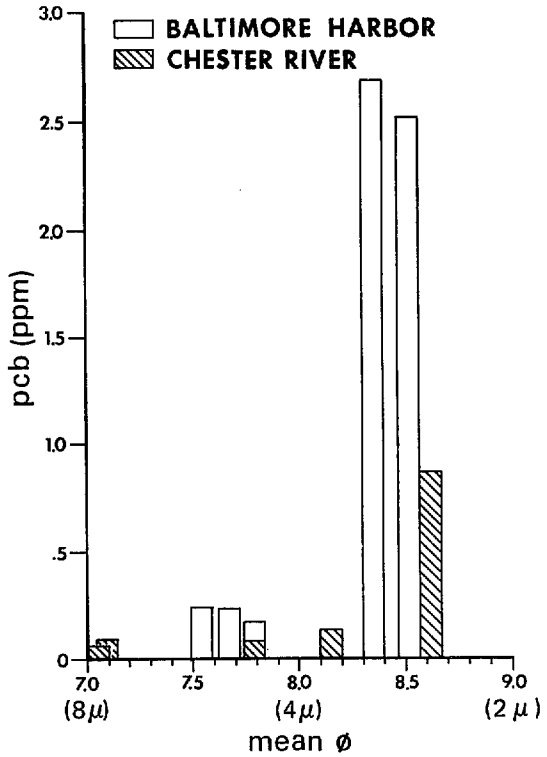

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Figure 4-25. Polychlorinated Biphenyl Concentrations in Bottom Sediments from Baltimore Harbor and the Chester River (depicted as a function of grain size.)

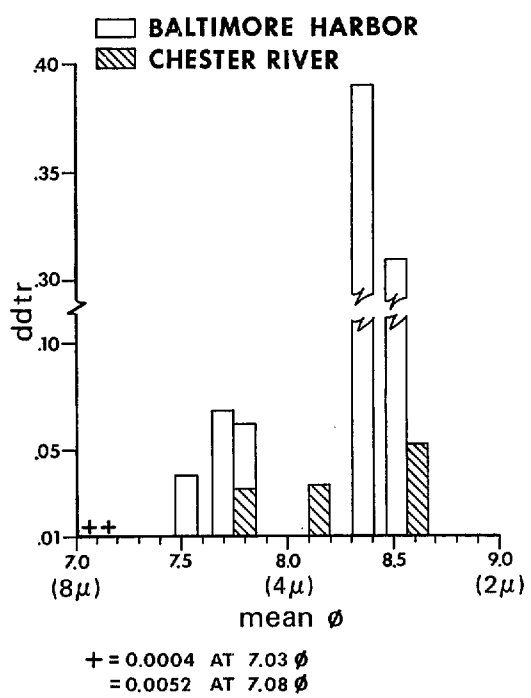

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Figure 4-26. DDT Residue Concentrations in Bottom Sediments from Baltimore Harbor and the Chester River (depicted as a function of grain size.)

4.1.6 Acknowledgements

The Upper Bay Survey required extensive field operations and long hours aboard ship. It is a pleasure to acknowledge the able support provided by Grey Lyons, Manu Swain, Jeff Nolder and Rick Woll. The tedious and difficult laboratory analyses were conducted by Tehana Merryman. Scanning electron microscope work was performed under the direction of Bob Wytkowski of the Westinghouse Research and Development Laboratory in Pittsburgh. The technical competence and dedication of all these assistants contributed to the successful completion of this aspect of the survey.

Editorial comments by M. Grant Gross and Jerry Schubel contributed to the final manuscript.

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4.2 Suspended Sediment in the Waters of the Upper Chesapeake Bay

4.2.1 Introduction

For the purposes of this discussion, the upper Chesapeake Bay is defined as the segment of the bay proper north of 38°57'N. It is generally shallow with a mean depth of nearly four meters. The hypsometry of the bay has recently been described by Cronin and Pritchard (1975). The bottom sediments are predominantly mud—silt and clay—except in the littoral zone where sand locally derived from erosion of the coast predominates (Ryan, 1953; Schubel, 1968a; Palmer, this volume).

The upper Chesapeake Bay is clearly the estuary of the Susquehanna River. The Susquehanna, entering at the head of the bay, is the only river that discharges directly into the main body of the Chesapeake Bay. All of the other rivers discharge into estuaries that are tributary to the bay proper. The Susquehanna, with a longterm average discharge of about 985 m^3/sec, supplies approximately 90 percent of the total freshwater input to the bay north of 39°57'N. The characteristic seasonal variation in the riverflow, high discharge in late winter and early spring followed by low to moderate flow throughout the summer and most of the fall, is revealed by an ensemble average taken by month from the Conowingo Dam records of 1929-1966 as shown in Figure 4-27 (Boicourt, 1969).

The Susquehanna flow regime and the associated circulation patterns generated within the upper Chesapeake Bay in response to the varying role of the river produce two distinctive distributions of suspended sediment and concomitant patterns of sediment transport. The first characterizes the spring freshet and other periods of very high riverflow. The second, characteristic of periods of low to moderate riverflow, typifies most of the remainder of the year. These are particularly evident in the upper 30 to 40 km of the bay. Farther seaward, the coupling of the distribution and transportation of suspended sediment to the discharge of the Susquehanna is less apparent.

4.2.2 Sources of Sediment

The ultimate sources of sediments to the upper Chesapeake Bay are rivers, shore erosion, and biological activity within the bay. An additional source of sediment to the upper bay—a proximate source—is the transport of sediment from more seaward segments of the estuary by the net upstream flow of the lower layer. Thus, the sources of sediment to the upper bay are external, internal, and marginal. The predominant source of fine-grained sediment is the fluvial input, and it is the only one considered in this report.

The Susquehanna River is the only significant source of fluvial sediment to the upper Chesapeake Bay. The sediment being discharged is predominantly clay and silt; the coarser particles are entrapped in the reservoirs along the lower reaches of the river. All of the other rivers debouch into estuaries, and the bulk of their sediment loads are trapped in the upper reaches of those tributaries. These tributaries probably act as sediment sinks rather than sources to the main body of the bay. Not only do they entrap most of the sediment introduced by their rivers, but they also entrap fine sediment carried into them from the bay proper, sediment derived primarily from the Susquehanna. This mechanism has been described on a number of occasions over the years by D.W. Pritchard (personal communication) and by Schubel (1968a, 1972a), and was demonstrated by the Chester River Study (Clarke et al., 1972).

During most years, the bulk of the sediment is discharged during the normal spring freshet when both riverflow and the concentration of suspended sediment are high. Since the spring of 1966, the Chesapeake Bay Institute has been monitoring the input of suspended sediment by the Susquehanna River by sampling the discharge at the Conowingo Hydroelectric Plant (Schubel, 1968b; 1971a; 1974). In the twelve-month period from April 1, 1966 through March 31, 1967, the Susquehanna discharged an estimated 0.6 x 10^6 metric tons of suspended sediment into the upper Chesapeake Bay (Schubel, 1968b). In 1969 the estimated input was approximately 0.3 x 10^6 tons (Schubel, 1971b). In 1972—the year of Tropical Storm Agnes—the Susquehanna discharged an estimated 33 x 10^6 tons of sediment into the upper bay, more than 95 percent of which was discharged in June following Agnes (Schubel, 1974).

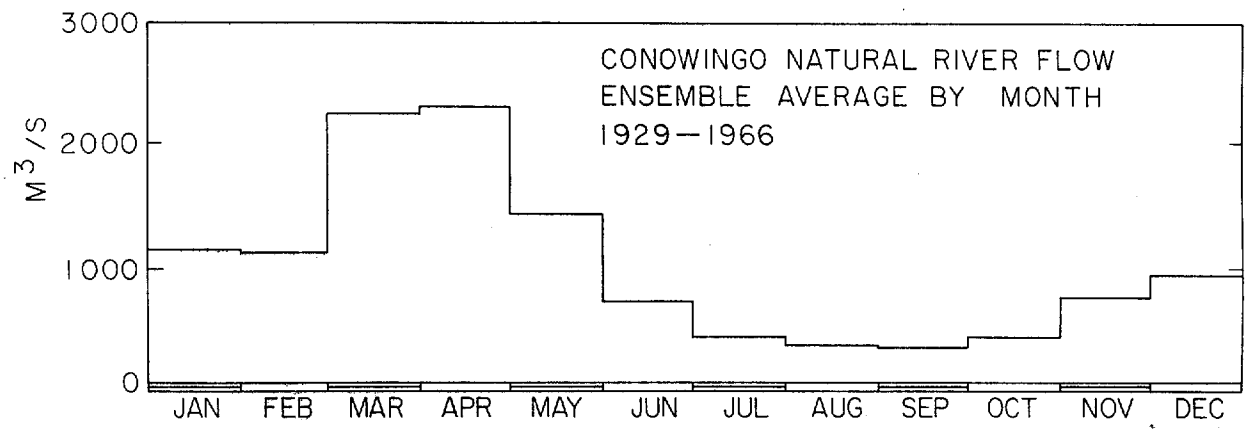

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Figure 4-27. Ensemble Average of the Susquehanna River at Conowingo, 1929-1966. (An estimate of the monthly streamflow entering the Chesapeake Bay is depicted in Figure 5-12, and the discharge of the Susquehanna River in 1973 and 1974 is shown in Figures 4-28 and 4-29.)

Estimates of the suspended sediment discharge of the Susquehanna River were made also for the period covered by this report. The same procedures were followed. Daily water discharge records for the Susquehanna River at Conowingo, Maryland, were furnished by the Conowingo Hydroelectric Plant. The data for 1973 and 1974 are plotted in Figures 4-28 and 4-29. During the same period, water samples were collected on nearly a daily basis from the tailrace of the hydroelectric plant for determining the concentrations of total suspended solids and of combustible organic matter. Water samples were collected with a specially-constructed, weighted, 1.2-liter water bottle that was lowered from the top of the dam through the discharge. The samples were filtered through preweighed 47 mm, 0.6 μm APD Nuclepore filters.* The filters were desiccated over silica gel at ambient temperature for at least 72 hours and reweighed, then the concentrations (mg/l) of total suspended solids were calculated. All weighings were made to $\pm$0.01 mg. The concentrations of total suspended solids during 1973-74 are plotted in Figures 4-30 and 4-31.

The suspended sediment discharge was estimated in the following way: Each suspended sediment concentration was multiplied by the mean daily water discharge averaged over the period (defined by the mid-points between sampling dates) and by the length of this time interval to obtain the mass of sediment discharged during that period. Next, these data were summed to generate the total mass of suspended sediment discharged by the Susquehanna River into the upper Chesapeake Bay during 1973-74. The suspended sediment discharge data are plotted in Figures 4-32 and 4-33 as the cumulative mass percent and as the mass of suspended sediment discharged each month.

The procedure described above results in an estimated discharge of 1.2×10^6 metric tons for 1973 and of 0.8×10^6 metric tons for 1974. A number of assumptions are involved in the determination of the estimated suspended sediment discharge. It was assumed that each suspended sediment concentration was representative of the entire mass of water being discharged at the time of sampling. This assumption is probably reasonable, because the suspended sediment is very fine-grained and because the samples were collected directly from the well-mixed discharge water of the Conowingo Hydroelectric Plant. It was assumed also that each concentration was representative of the time interval, one to two days, defined by the midpoints between sampling dates. This assumption is reasonable except when water discharge may change markedly in a short time. The other assumptions are incorporated in the averaging which is implicit in the calculation. To evaluate these assumptions, the calculation will be examined more closely. The following terms are defined:

D_i = the mass of sediment discharged during a time interval Δt_i.

t_i = the time interval defined by the midpoints between sample dates.

D_E = the estimated mass of sediment discharged during the year.

$\bar{C}_i$ = the average concentration of suspended sediment over the time interval Δt_i.

C_i = the measured instantaneous suspended sediment concentration at the specific time t within the time interval Δt_i.

C = the instantaneous suspended sediment concentration averaged over the cross-section of discharging water.

R = the instantaneous water discharge.

$\bar{R}_i$ = the mean water discharge averaged over the time interval Δt_i.

* Nuclepore polycarbonate filters are produced by General Electric, and are distributed by Arthur H. Thomas and other scientific supply houses. (APD is average pore diameter.)

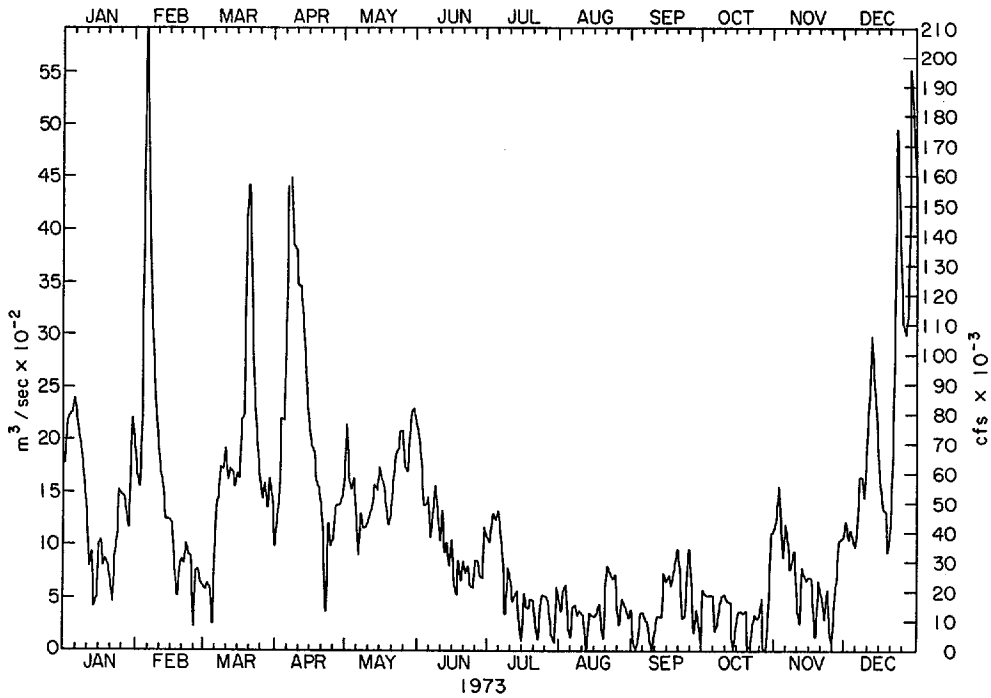

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Figure 4-28. Discharge of the Susquehanna River at Conowingo in 1973.

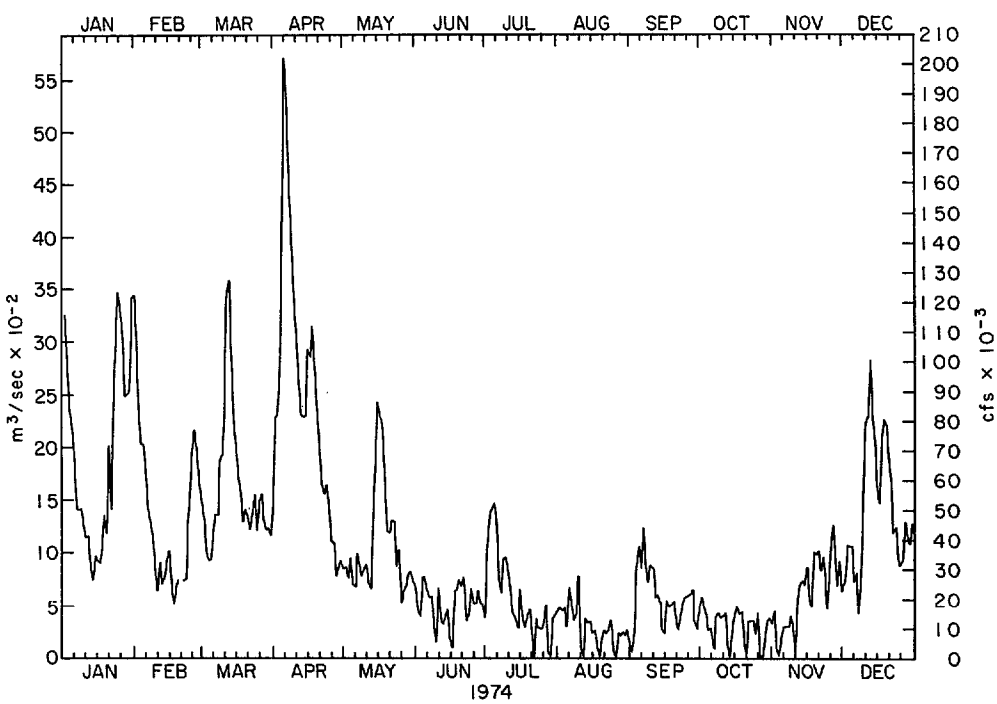

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Figure 4-29. Discharge of the Susquehanna River at Conowingo in 1974.

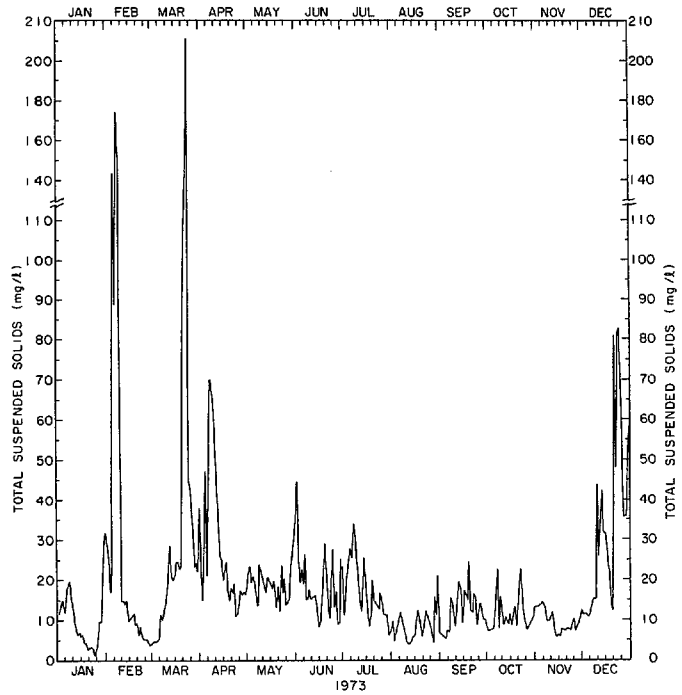

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Figure 4-30. Concentration (mg/l) of Suspended Sediment in the Susquehanna River (on the downstream side of the Conowingo Hydroelectric Plant) During 1973.

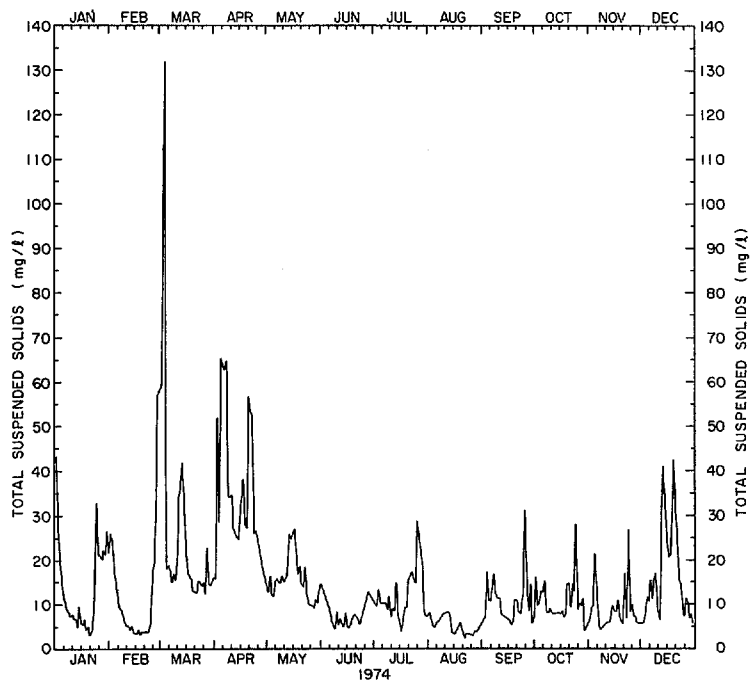

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Figure 4-31. Concentration (mg/l) of Suspended Sediment in the Susquehanna River (on the downstream side of the Conowingo Hydroelectric Plant) During 1974.

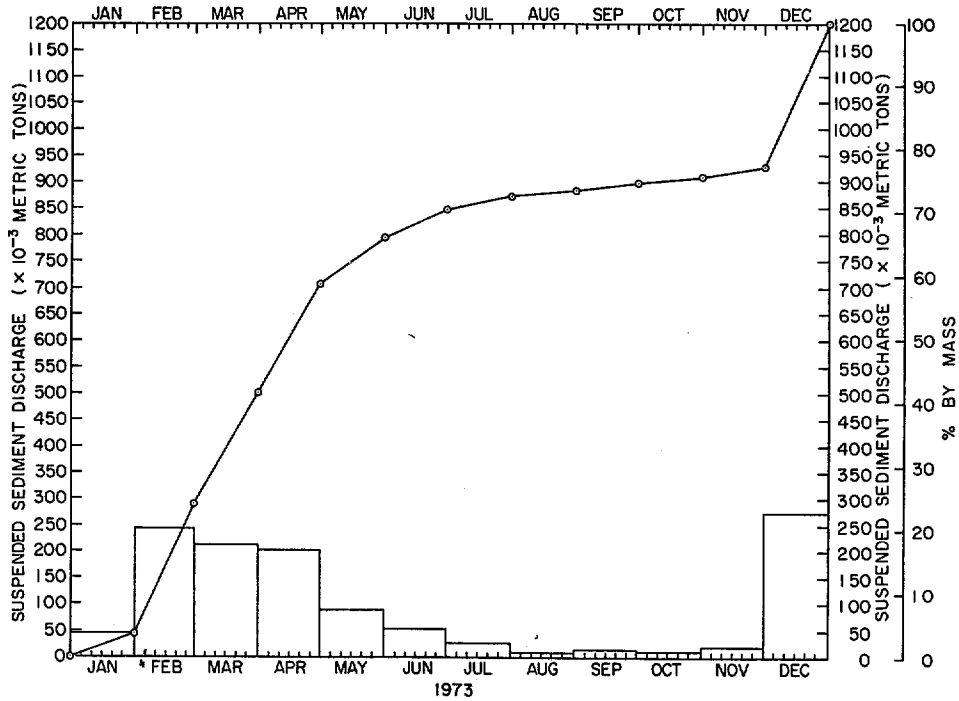

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Figure 4-32. Suspended Sediment Discharge of the Susquehanna River at Conowing During 1973 (plotted as cumulative mass percent and as the mass discharged during each month).

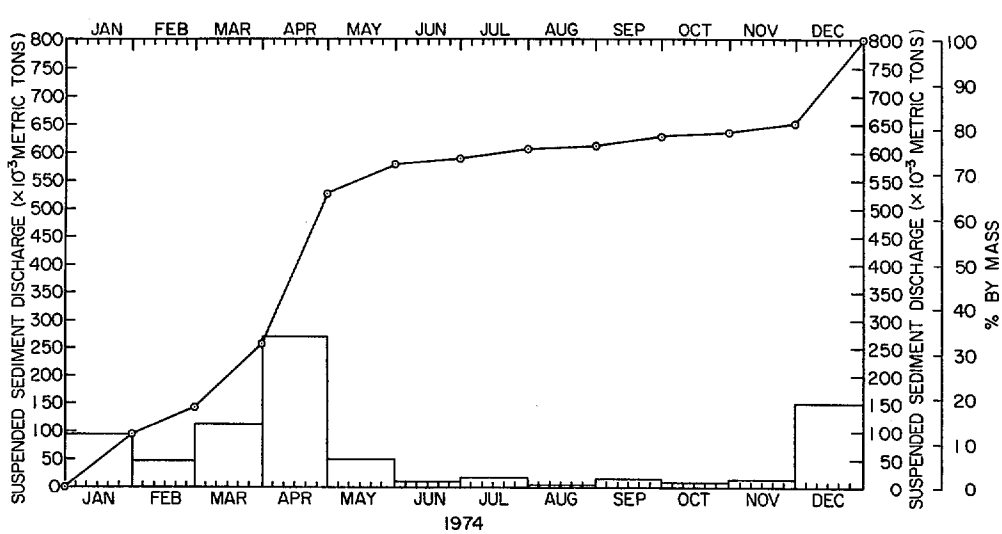

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Figure 4-33. Suspended Sediment Discharge of the Susquehanna River at Conowingo During 1974 (plotted as cumulative mass percent and as the mass of sediment discharged each month).

The mass of suspended sediment discharged over the time interval, T, of one year where

$$T = \sum_{i=1}^{n} \Delta t_i$$

was estimated by

$$D_E = \sum_{i=1}^{n} D_i = \sum_{i=1}^{n} C_i \bar{R}_i \Delta t_i. \tag{1}$$

Equation (1) also may be written as

$$D_E = \sum_{i=1}^{n} \bar{C}_i \bar{R}_i \Delta t_i + \sum_{i=1}^{n} (C_i - \bar{C}_i) \bar{R}_i \Delta t_i \tag{2}$$

The true value of the sediment discharged over the time interval T may be expressed by

$$D_T = \sum_{i=1}^{n} (\overline{CR})_i \Delta t_i \tag{3}$$

where

$$(\overline{CR})_i = \frac{1}{\Delta t_i} \int_{\Delta t_i} CRdt$$

The instantaneous values of C and R can be expressed as the sum of a mean value and mean deviation. Thus, for the time interval, Δt, one can write

$$C = \bar{C}_i + C'_i$$
$$R = R_i + R'_i$$

If one takes the product of these and averages, he obtains

$$(\overline{CR})_i = \overline{\bar{C}_i \bar{R}_i} + \overline{C'_i \bar{R}_i} + \overline{\bar{C}_i R'_i} + \overline{C'_i R'_i};$$

which reduces to

$$(\overline{CR})_i = \bar{C}_i \bar{R}_i + \overline{C'_i R'_i} \tag{4}$$

The terms $\overline{C'_i \bar{R}_i}$ and $\overline{\bar{C}_i R'_i}$ both equal zero, since $\bar{R}'_i = \bar{C}'_i = 0$. And it is obvious that $\bar{C}_i \bar{R}_i = \bar{C}_i \bar{R}_i$ if one averaged over the same time interval used to define $\bar{C}_i$ and $\bar{R}_i$. The term $\overline{C'_i R'_i}$ does not equal zero however, except under very special circumstances or unless the variables are uncorrelated (which is not the case here).

Using Equation (4), Equation (3) can be rewritten as

$$D_T = \sum_{i=1}^{n} \bar{C}_i \bar{R}_i \Delta t_i + \sum_{i=1}^{n} C'_i R'_i \Delta t_i. \tag{5}$$

The difference between the true suspended sediment discharge (5) and the estimated discharge (2) is then $D_T - D_E$ or

$$D_T - D_E = \sum_{i=1}^{n} \overline{C'_i R'_i} \Delta t_i - \sum_{i=1}^{n} (C_i - \bar{C}_i) \bar{R}_i \Delta t_i. \tag{6}$$

The error in the estimate (2) of the mass of suspended sediment discharged during the time interval, T, then is given by the difference of two terms. The first term on the right side of Equation (6) depends on the correlation between the fluctuations of the suspended sediment concentration and the water discharge during the time interval Δt_i. The suspended sediment concentration was generally measured by daily intervals; thus, one assumption implicit in the calculations used here is that the correlation of the fluctuations of river discharge and suspended sediment concentration at frequencies higher than one per day may be ignored. In general, the concentration of suspended sediment increased

with river discharge and therefore, the correlation between them is positive. The first term on the right of Equation (6), then, is positive, and our estimate of the suspended sediment discharge, D_E, tends to be less than the true value, D_T.

The second term on the right side of Equation (6) depends on the difference between a single determination of the suspended concentration taken at a specific time t during the time interval Δt_i and the mean value of the suspended sediment concentration over the time interval Δt_i. This term may be either positive or negative; hence, it may either add or subtract from the bias introduced into the estimate by the first term. Since there were 316 suspended sediment determinations in 1973 and 346 determinations in 1974, and since there is equal probability that $(C_i - \bar{C}_i)$ for any single set will be either positive or negative, the effect of this term on the estimate is probably quite small. It follows that the calculations of 1.2×10^6 metric tons for 1973 and 0.8×10^6 metric tons for 1974 are probably underestimates, but it cannot be said by how much. The estimates could not have been improved easily. Sampling at frequencies more often than once per day does not seem warranted, because only daily mean water discharge records are available.

4.2.3 Suspended Sediment Population

At any point in time and space, Chesapeake Bay's suspended sediment is a subpopulation of the bay's total sediment population. It is made up of newly introduced inorganic sediment from rivers and shore erosion which has not been deposited; of living plankton; of organic detritus which has not settled out; and of previously deposited organic and inorganic sediments which have been resuspended from the bay's floor. At any given time, all of these components are present, but their relative abundances vary both temporally and spatially.

The inputs of new sediment have already been described. Schubel (1968a) demonstrated the importance of a proximate source of sediment–the resuspension of bottom sediments by tidal scour — to the suspended sediment population of the upper 20 to 30 km of the bay. Subsequent observations have confirmed the importance of this process in determining the concentrations of total suspended solids, particularly in the upper 20 to 30 km of the estuary, where depths are shallow and tidal mixing is intense. Farther seaward, the effects of resuspension are observable only near the bottom.

4.2.4 Distributions of Temperature, Salinity, and Total Suspended Solids

4.2.4.1 Temperature and Salinity

Conductivities and temperatures were determined with a Chesapeake Bay Institute Induction-Conductivity-Temperature-Indicator (ICTI). These measurements have a precision and accuracy of ±0.02°C in temperature and ±0.02 milliohms per centimeter in conductivity. Salinities, calculated from the temperature and conductivity data, have an accuracy and precision of ±0.03°/oo.

The distributions of temperature and salinity in the upper Chesapeake Bay at approximately monthly intervals from December 1973 to November 1974 are plotted in Figures 1 thru 27 in Volume III, Appendix B. The station locations are the same as those described in other sections of this report. The primary purpose of collecting these data was for input to the numerical model (Hunter, Volume IV).

4.2.4.2 Total Suspended Solids

Measured volumes of water, collected with a submersible pump, were filtered through preweighed 47 mm, 0.6μ pore diameter nuclepore filters. Volumes were determined to the nearest three milliliters, and usually, approximately 500 ml of water were filtered. The filters were rinsed a number of times to remove any sea salt, placed in individual desiccators, and desiccated over silica gel at ambient temperature for at least 72 hours. After drying, the filters and their sediment loads were reweighed, and the concentrations of total suspended solids were calculated. All weighings were made to ±0.01 mg. Selected samples were combusted at 500°C for 30 minutes and re-weighed to determine the

loss of mass after combustion. These data provide the concentrations of combustible organic matter, and they are most commonly reported as the percent of the total concentrations accounted for by combustible organic matter.

The distributions of total suspended solids (suspended sediment) in the upper Chesapeake Bay at approximately monthly intervals from October 1973 to November 1974 are shown in Figures 28 through 45 in Volume III, Appendix B. The station locations are the same as those described in earlier sections of this report.

The primary objective of collecting these data was to provide input to the numerical model described elsewhere in this report (Hunter, Volume IV). However, a general description of the processes that control the distributions of suspended sediment in the upper bay is included for completeness. This discussion is based not only on observations made during this study, but on the vast body of data gathered over the past ten years in studies supported by the State of Maryland.

The Susquehanna River discharges nearly 90 percent of the total freshwater input to the upper Chesapeake Bay, as defined in this report. As pointed out previously, the upper bay is clearly the estuary of the Susquehanna River, which is the only significant supplier of freshwater and also of fluvial sediment. During the spring freshet and other short periods of very high riverflow, the suspended particle population is closely linked to the ultimate source of most of the new sediment, the Susquehanna. During these periods there is a downstream gradient of the concentration of total suspended solids (suspended sediment).

At other times of the year, however, the concentrations of suspended sediment typically are higher in the upper 30 km of the estuary (Tolchester to Turkey Point) than farther upstream in the source river, or farther seaward in the estuary. During these periods, the internal sediment sources-primary productivity, shore erosion, and the resuspension of bottom sediments by tidal scour—play a more important role. The significance of tidal resuspension is particularly evident in the bay from Tolchester to Turkey Point where, coupled with the net non-tidal estuarine circulation, it produces the *turbidity maximum* (Schubel, 1968c). Sediment discharge by the Susquehanna is relatively less important but still significant.

In more seaward segments of the upper bay, the effects of tidal resuspension are apparently only near the bottom. The distribution of suspended sediment away from the bottom is controlled largely by primary productivity, the introduction of new fluvial sediment by the Susquehanna, and the escape of fine resuspended sediments from the zone of the turbidity maximum.

Schubel (1968a, b; 1969; 1972a, b) has discussed in some detail the processes that control the distribution and transportation of suspended sediment in the upper 30 km of the Chesapeake Bay (Turkey Point to Tolchester). Biggs (1970) has described the sources and distribution of suspended sediment in the upper bay from its head at Turkey Point to seaward of the Bay Bridge at Annapolis. For a thorough discussion the reader is referred to these publications.

4.2.5 Size Distribution of Suspended Particles

4.2.5.1 Methods

The particle size distributions of the suspended particles were determined with a photomicrographic Zeiss Particle Size Analyzer TGZ-3. The technique consists of measuring the particle images on photomicrographs with the semi-automatic Zeiss analyzer. The entire procedure involves four steps: (1) sample collection (2) slide preparation, (3) photography of the sample, and (4) measurement of the particle images with the Zeiss Particle Size Analyzer.

Sample Collection - Water samples collected with a submersible pump were immediately filtered through 0.22μ APD Millipore filters. After filtration, the filters and sediment were washed several times with distilled water to remove any sea salt and stored in a desiccated box. Several volumes were filtered at each sample depth to help ensure the proper density of particles on the filter surface. For microscopic size analyses the ideal sample is a single-particle layer with no particle touching another.

Slide Preparation - The filters were cleared (rendered transparent) by impregnating them with a liquid having the same index of refraction (n=1.510). An appropriate mix, determined empirically, of Zeiss mounting media was used.

Photography of Sample - The cleared filters and their sediment loads were photographed with a phase microscope. Phase microscopy was used to enhance the visibility of the particles, many of which have little color and have a refractive index near 1.51; hence they are nearly invisible under ordinary light. The photography was done on a Zeiss Standard Universal Microscope equipped with a bright-field, phase-contrast, dark-field condenser having a numerical aperture of 1.4. The light source, a 6V-15W lamp built into the microscope, was adjusted for Köhler illumination. The light was filtered with a green interference filter having maximum transmission at 546 mμ to increase resolving power. A Neoflour Ph 40/0.75 objective was the working objective. The objective was used in conjunction with an 8X eyepiece, and with the Optovar set at 1.50. Thus, the approximate observed magnification was 40 x 8 x 1.5 = 480. The camera factor was 0.5X, hence the image magnification was about 240. The negatives were enlarged to produce a total magnification of 2000X. This final magnification was carefully adjusted to this value by projecting an image of a stage micrometer photographed on each roll of film and adjusting the enlarger until the desired magnification was obtained. The fine-grain film, Kodak Pan X, was developed in Microdol developer. The 40X objective has a theoretical useful magnification of about 1500; therefore, a 2000X enlargement contains some empty magnification. Previous analyses with a 100X objective having a theoretical useful magnification of 2500X (Schubel, 1968a) indicate that the empty magnification resulting from the procedures used in this study does not falsify the determination of the particle size distributions. The upper limit of useful magnification is a theoretical limit, not a practical one.

The smallest particle image that can be measured with the Zeiss Particle Size Analyzer in the mode employed was 1.2 mm. With a magnification of 2000X this corresponds to a particle diameter of 0.6μ.

Fields that were photographed were selected at random. Generally 10 to 20 photographs were taken of each sample. Examples of typical photomicrographs are shown in Figures 4-34 through 4-36.

Particle Size Measurement - The particle images on the photomicrographs were sized with a Zeiss Particle Size Analyzer TGZ-3, a semi-automatic device in which the eye and judgement of the operator participate in the measuring process. The principal components of the instrument are a light source, a lens system, and an adjustable iris diaphragm. This diaphragm is correlated via a commutator with 48 telephone counters, each counter corresponding to a certain operative interval of the iris diaphragm. The instrument is equipped also with a cumulative counter which registers the total number of particles measured. The iris diaphragm is illuminated from below and is imaged as a sharply defined circular light spot in the plane of the plexiglass plate that supports a photomicrograph. The photomicrograph is moved by hand until the center-of-area of the particle's image lies approximately at the center of the measuring mark. The particle image is then measured by adjusting the diaphragm until the light spot has an area equal to that of the particle image. For irregular particles one attempts to adjust the diameter of the spot so that the total area of those parts of the particle protruding beyond the measuring mark is equal to the re-entrant areas. Once the diaphragm is appropriately adjusted, the foot switch is depressed. This activates the proper counter; a hole is automatically punched into the particle image, and the total registered on the cumulative counter is increased by one. The photomicrograph is shifted then to another particle image, and the process is repeated. For each sample depth, 1,500 to 2,000 particles were measured. An experienced operator can measure approximately 1,000 particles in 30 to 45 minutes. A sample of 1,000 particles was found to be adequate to secure agreement between pairs of the mean and standard deviation calculated on successive cycles of sample size doubling to within 15 percent of the average of each pair of values.

The particle size measured by this procedure is obviously an equivalent diameter-the diameter of a circle having an area equivalent to that of the projected area of the particle in question. This diamter is defined as D_m. The measure of size required for the numerical model is the average settling velocity of the particles; a value much more difficult to determine than D_m. The settling velocity (size) distribution of a population of fine particles is most commonly determined by a sedimentation analysis.

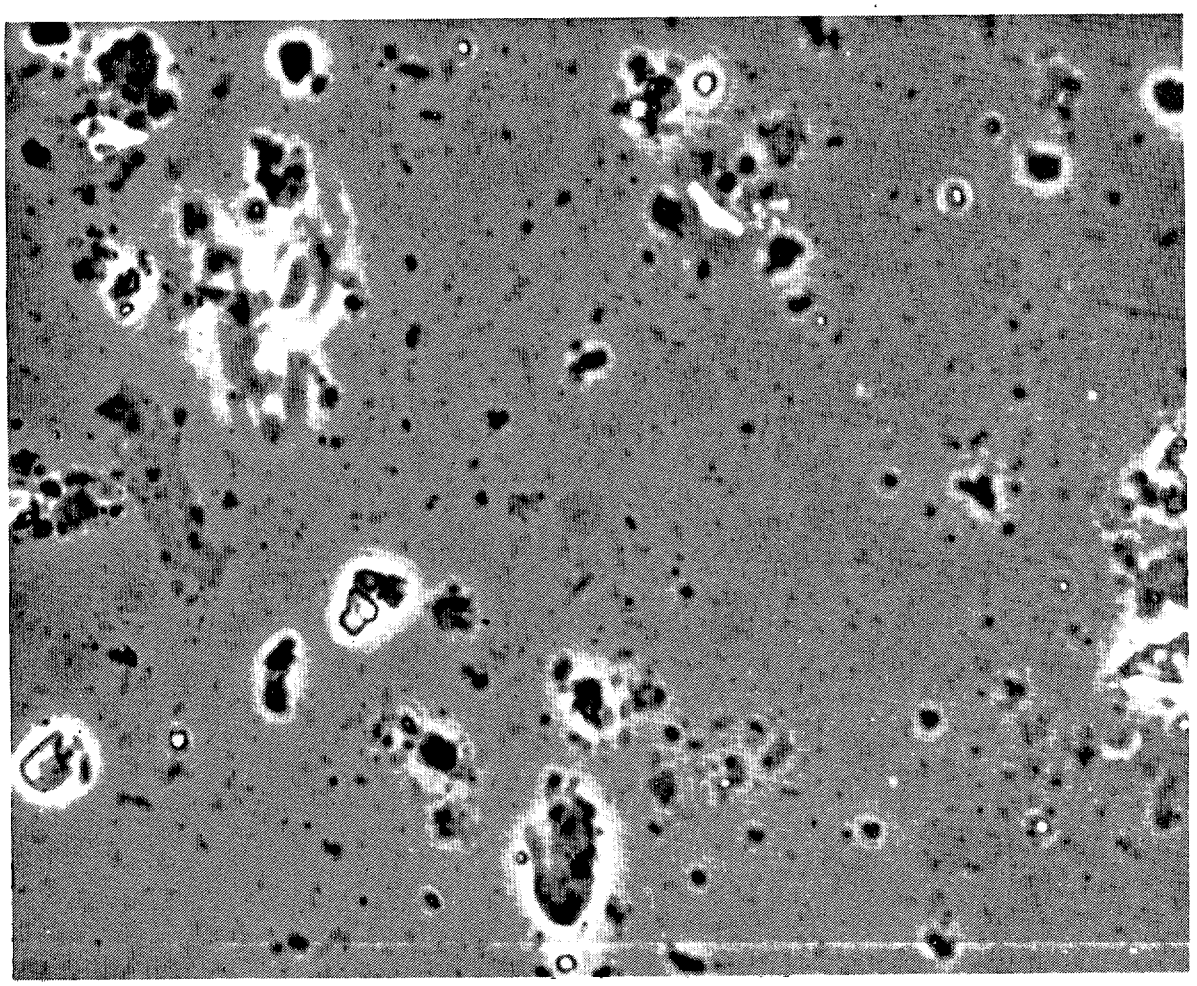

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Figure 4-34. Photomicrograph of Sample of Suspended Sediment from Upper Chesapeake Bay. (The sample was collected on a Millipore filter, cleared with cedar oil, and photographed using phase microscopy. Total magnification 2000X.)

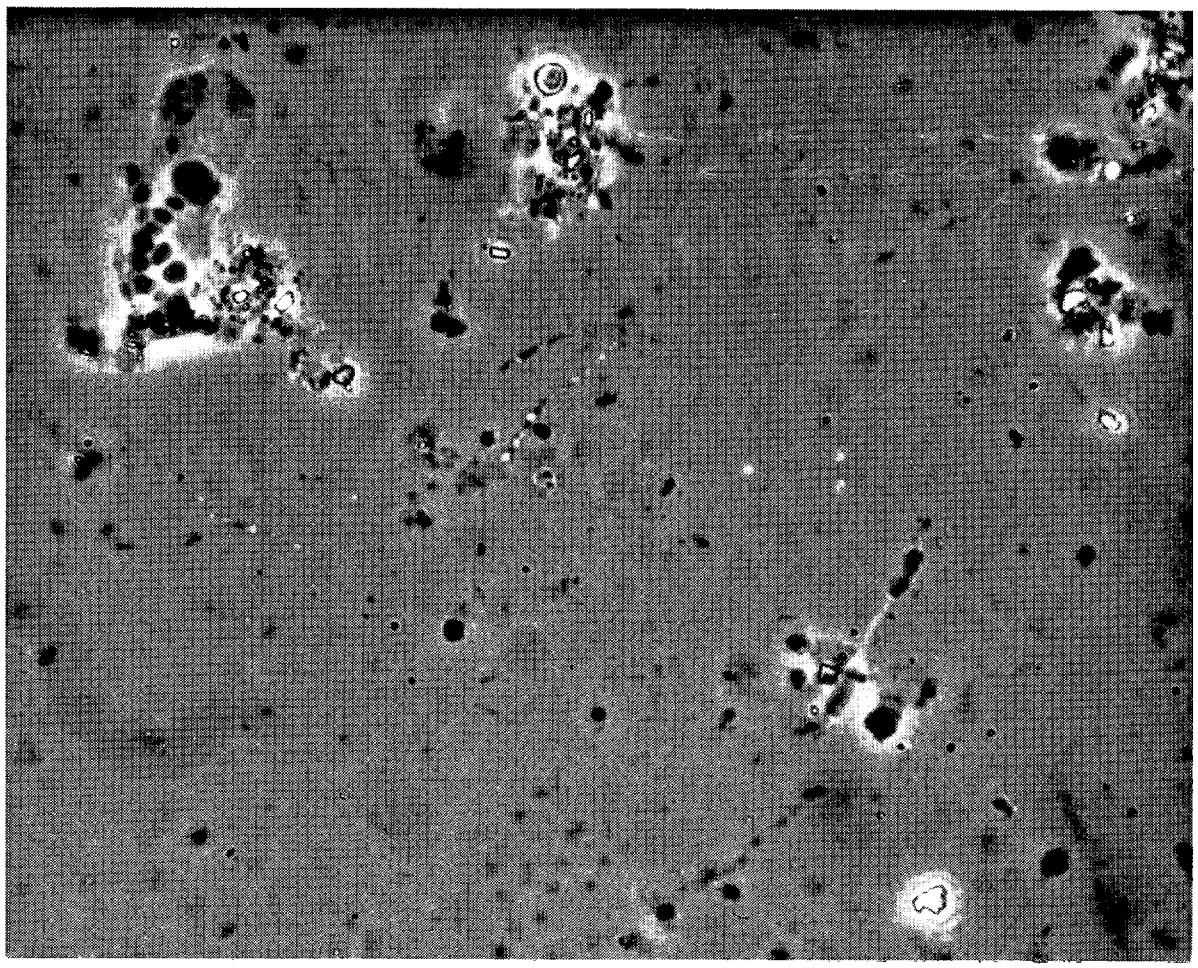

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Figure 4-35. Photomicrograph of Sample of Suspended Sediment from Upper Chesapeake Bay. (The sample was collected on a Millipore filter, cleared with cedar oil, and photographed using phase microscopy. Total magnification 2000X.)

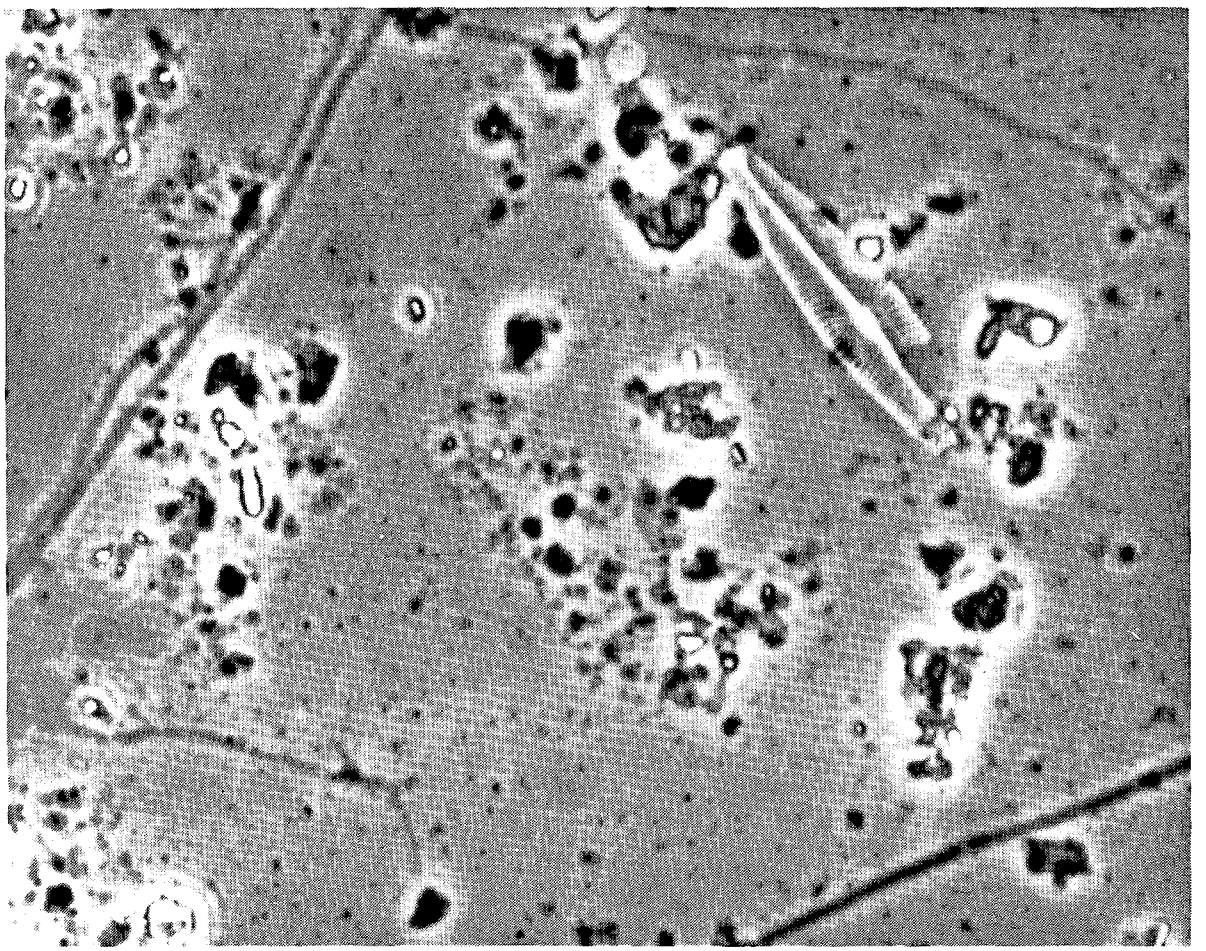

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Figure 4-36. Photomicrograph of Sample of Suspended Sediment from Upper Chesapeake Bay. (The sample was collected on a Millipore filter, cleared with cedar oil, and photographed using phase microscopy. Total magnification 2000X.)

The results of the analysis generally are expressed in terms of an equivalent diameter (D_s)—the diameter of a sphere with a density equal to that of the particle and having a settling velocity equivalent to that of the particle. For any given particle, D_s and D_m need not be equal, and they seldom are. D_s is frequently referred to as a *Stokes' diameter,* but in fact, it is a *velocity*. Two particles having the same D_s will settle with the same speed in a fluid. Their shapes, surface areas, cross-sectional areas, projected diameters, and volumes may differ markedly, as may any of their orthodox statistical measures of size.

We have attemped to estimate characteristic settling velocities for the suspended particle population of the upper Chesapeake Bay in a variety of ways: (1) by calculations from Stokes' law, using measured values of D_m and particle density, (2) by direct measurement of the settling velocity size distribution with a Mine Safety Appliance Particle Size Analyzer (Schubel, 1968a), and (3) by calculation on theoretical grounds.

4.2.5.2 Results

Number-Size Distributions - The number-size distributions were determined for samples of suspended sediment from three depths—surface, mid-depth, and one meter above the bottom—at 10 to 13 stations during each of four seasons—December 1973, March 1974, June 1974, and September 1974. The data for each of the individual distributions are included in the Upper Bay Survey Data Base (Volume III) and are not repeated in this chapter. The Data Base also contains the volume-transformations of the number-size data. Several representative number-size distributions are plotted in Figures 4-37 and 4-38. Each of the these figures depicts the percent (by number) of particles with equivalent projected diameters (D_m) greater than any particular size indicated on the abscissa.

The statistical properties of all the number-size distributions are summarized in Table 4-1. In calculating these statistics, the 48 size classes of the analyzer and the classes added with a template to cover the larger particles were grouped by threes to form 25 classes. The statistics calculated are standard moment measures. The mean is the first moment about zero, and the standard deviation is the square root of the second moment about the mean. The skewness and kurtosis are defined in terms of the second ($\mu 2$), third ($\mu 3$), and fourth ($\mu 4$) moments by

$$\text{Skewnes} = \tfrac{1}{2}\frac{\mu_3}{\mu_2^{3/2}}$$

$$\text{Kurtosis} = \frac{\mu_4}{\mu_2^{2}}$$

The 25-class midpoint, $X\nu$, used in the calculations is defined by

$$X\nu \qquad X_\nu = \frac{\xi_{3\nu-2}\, f_{3\nu-2} + \xi_{3\nu-1}\, f_{3\nu-1}\, f_{3\nu-1} + \xi_{3\nu}\, f_{3\nu}}{f_{3\nu-2} + f_{3\nu-1} + f_{3\nu}}$$

which reduces to

$$X\nu = \frac{\sum_{i=3\nu-2}^{3\nu} \xi_i f_i}{\sum_{i=3\nu-2}^{3\nu} f_i}$$

where ξ_i are the mid-points of the 75 sub-classes, f_i the frequency of observations in each sub-class, and $\nu = 1, \ldots 20$.

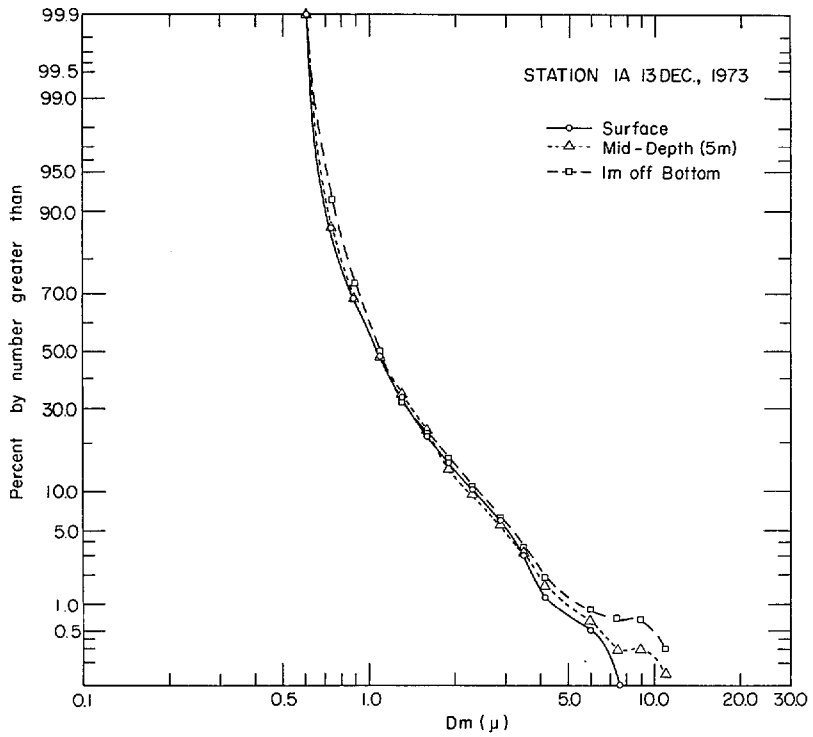

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Figure 4-37. Photomicrographically Determined Number-Size Distributions of Suspended Sediment (from three depths at Station 1A on December 13, 1973).

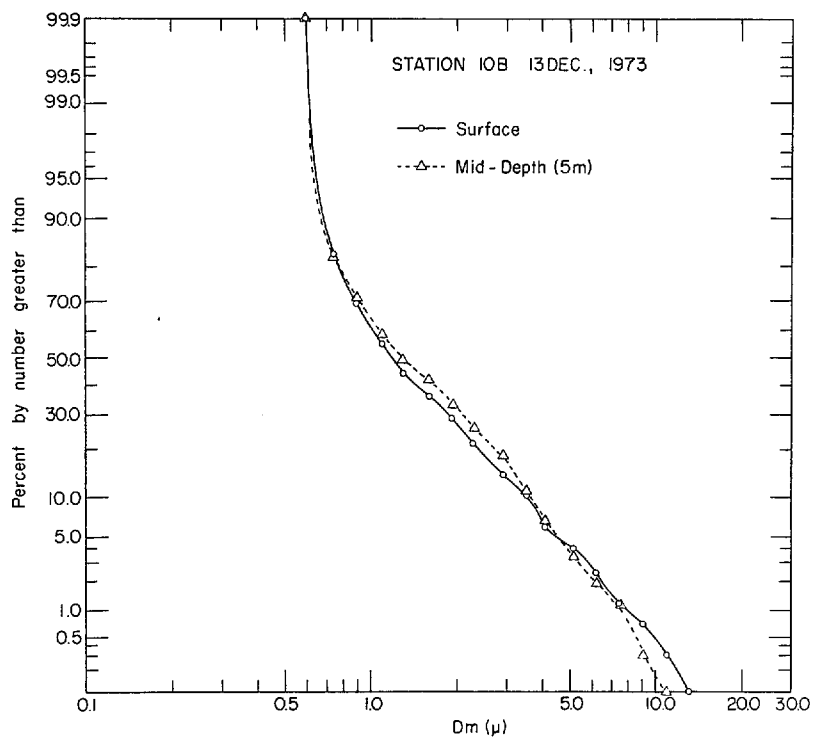

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Figure 4-38. Photomicrographically Determined Number-Size Distributions of Suspended Sediment (from two depths at Station 10B on December 13, 1973).

TABLE 4-1. STATISTICAL PROPERTIES OF THE NUMBER-SIZE DISTRIBUTIONS OF THE SUSPENDED PARTICLE POPULATION OF THE UPPER CHESAPEAKE BAY

Date	Depth	Mean (μ)	Standard Deviation (μ)	Skewness	Kurtosis
Station 1A:					
Dec 13, 73	Surface	1.3	0.8	1.2	7.2
	Mid-Depth	1.3	0.9	2.3	37.4
	Bottom	1.4	1.1	3.5	86.8
Mar 21, 74	Surface	1.5	2.0	5.2	156.8
	Mid-Depth	1.4	1.3	3.1	74.5
	Bottom	1.4	1.2	2.2	29.7
Jun 13, 74	Surface	1.6	2.5	4.7	117.9
	Mid-Depth	1.4	1.1	1.5	12.9
	Bottom	1.9	1.5	1.6	15.0
Sep 20, 74	Surface	1.4	1.7	3.3	54.3
	Mid-Depth	1.5	1.4	2.8	61.3
	Bottom				
Station 2B:					
Dec 14, 73	Surface	1.4	0.8	1.2	9.2
	Mid-Depth	1.4	0.8	1.4	12.0
	Bottom	1.5	0.9	1.6	15.8
Mar 20, 74	Surface	1.2	0.9	3.7	126.8
	Mid-Depth	1.3	1.0	1.1	5.7
	Bottom	1.3	1.1	1.9	19.5
Jun 13, 74	Surface	1.9	1.7	1.7	14.2
	Mid-Depth	1.6	1.2	1.4	9.9
	Bottom	1.6	1.4	1.7	16.1
Sep 20, 74	Surface	1.8	2.0	2.4	34.7
	Mid-Depth	1.6	1.7	1.9	18.7
	Bottom	1.8	2.1	2.4	32.4

TABLE 4-1. STATISTICAL PROPERITIES OF THE NUMBER-SIZE DISTRIBUTIONS OF THE SUSPENDED PARTICLE POPULATION OF THE UPPER CHESAPEAKE BAY (Continued)

Date	Depth	Mean (μ)	Standard Deviation (μ)	Skewness	Kurtosis
Station 3B:					
Dec 14, 73	Surface	1.3	1.1	5.0	200.7
	Mid-Depth	1.3	0.9	1.8	18.8
	Bottom	1.3	0.9	1.3	10.2
Mar 20, 74	Surface	1.6	1.4	1.6	14.2
	Mid-Depth	1.5	1.5	2.2	34.1
	Bottom	1.3	1.2	1.9	20.5
Jun 13, 74	Surface				
	Mid-Depth	1.5	1.1	1.4	12.3
	Bottom	1.6	1.3	1.5	13.4
Sep 20, 74	Surface	1.4	1.3	2.0	23.5
	Mid-Depth	1.6	1.4	1.9	20.8
	Bottom	1.8	1.5	1.3	8.6
Station 4B:					
Dec 14, 73	Surface	1.6	1.2	2.1	33.3
	Mid-Depth	1.4	1.1	2.1	30.3
	Bottom	1.4	0.7	1.1	6.4
Mar 20, 74	Surface	1.4	1.2	2.2	34.8
	Mid-Depth	1.2	0.9	1.6	14.0
	Bottom	1.4	1.4	3.3	71.3
Jun 13, 74	Surface	1.4	1.3	1.7	16.8
	Mid-Depth	1.6	1.3	1.4	10.8
	Bottom	1.6	1.3	1.5	12.4
Sep 20, 74	Surface	1.7	1.4	1.8	22.7
	Mid-Depth	1.5	1.3	1.9	20.0
	Bottom	1.8	1.8	3.5	99.7

TABLE 4-1. STATISTICAL PROPERTIES OF THE NUMBER-SIZE DISTRIBUTIONS OF THE SUSPENDED PARTICLE POPULATION OF THE UPPER CHESAPEAKE BAY (Continued)

Date	Depth	Mean (μ)	Standard Deviation (μ)	Skewness	Kurtosis
Station 5A:					
Mar 21, 74	Surface	1.3	1.0	1.7	17.1
	Mid-Depth	1.3	1.0	2.4	45.4
	Bottom	1.3	1.1	2.2	30.3
Jun 13, 74	Surface	1.4	1.0	1.3	8.4
	Mid-Depth	2.0	2.0	2.0	23.0
	Bottom	1.7	1.6	1.4	10.3
Station 5B:					
Dec 14, 73	Surface	1.7	1.2	1.7	21.9
	Mid-Depth	1.4	0.8	1.0	4.6
	Bottom	1.5	1.0	1.0	5.1
Mar 21, 74	Surface	1.1	1.7	10.3	523.4
	Mid-Depth	1.3	1.1	1.9	19.2
	Bottom	1.5	1.7	2.4	37.7
Jun 13, 74	Surface	1.4	1.4	3.4	65.6
	Mid-Depth	1.6	1.8	3.8	88.2
	Bottom	1.7	1.6	1.8	17.1
Sep 20, 74	Surface	1.4	1.0	1.6	15.5
	Mid-Depth	1.6	1.3	1.4	10.4
	Bottom	2.0	2.0	2.1	31.0
Station 6C:					
Dec 14, 73	Surface	1.5	1.1	1.3	9.2
	Mid-Depth	1.3	0.9	1.5	14.1
	Bottom	1.5	1.0	2.0	41.3
Mar 20, 74	Surface	1.4	1.0	1.5	11.9
	Mid-Depth	1.6	1.2	1.6	19.2
	Bottom	1.3	1.2	2.2	29.4
Jun 13, 74	Surface	1.6	1.5	3.7	81.0
	Mid-Depth	2.2	1.9	3.6	101.2
	Bottom	2.5	1.8	1.5	12.3
Sep 20, 74	Surface	1.5	1.6	3.0	55.8
	Mid-Depth	1.7	1.5	1.6	12.8
	Bottom	2.6	2.9	1.7	18.0

TABLE 4-1. STATISTICAL PROPERTIES OF THE NUMBER-SIZE DISTRIBUTIONS OF THE SUSPENDED PARTICLE POPULATION OF THE UPPER CHESAPEAKE BAY (Continued)

Date	Depth	Mean (μ)	Standard Deviation (μ)	Skewness	Kurtosis
Station 7A:					
Dec 13, 73	Surface	1.2	0.7	1.2	7.7
	Mid-Depth	1.2	0.8	1.5	12.7
	Bottom	1.3	0.8	1.2	11.0
Mar 21, 74	Surface	1.4	1.0	1.5	14.9
	Mid-Depth	1.3	0.9	2.2	36.0
	Bottom	1.4	1.2	3.4	99.8
Sep 18, 74	Surface	1.4	1.0	1.4	9.9
	Mid-Depth	1.6	1.3	3.6	104.4
	Bottom	2.0	1.7	1.6	16.0
Station 8B:					
Dec 14, 73	Surface	1.3	0.9	1.6	20.8
	Mid-Depth	1.5	1.1	1.2	7.1
	Bottom	1.7	1.5	1.2	7.3
Station 8C:					
Dec 12, 73	Surface	1.8	1.2	2.9	78.0
	Mid-Depth	1.6	1.1	1.3	10.8
	Bottom	2.1	1.8	1.5	18.3
Mar 20, 74	Surface	1.3	0.9	1.4	13.6
	Mid-Depth	1.5	1.1	1.6	17.2
	Bottom	1.5	1.4	2.0	28.7
Jun 13, 74	Surface	2.1	1.8	2.4	50.1
	Mid-Depth	1.4	1.0	1.8	23.7
	Bottom	1.8	1.3	1.7	18.3
Sep 18, 74	Surface	1.3	1.2	2.0	25.1
	Mid-Depth	2.0	1.9	1.5	13.9
	Bottom	1.7	1.6	1.7	16.7

TABLE 4-1. STATISTICAL PROPERTIES OF THE NUMBER-SIZE DISTRIBUTIONS OF THE SUSPENDED PARTICLE POPULATION OF THE UPPER CHESAPEAKE BAY (Continued)

Date	Depth	Mean (μ)	Standard Deviation (μ)	Skewness	Kurtosis
Station 9A:					
Dec 14, 73	Surface	1.6	1.2	1.4	12.7
	Mid-Depth	1.9	1.3	1.2	12.4
	Bottom	2.0	1.5	1.2	8.1
Mar 21, 74	Surface	1.4	1.1	1.5	12.6
	Mid-Depth	1.7	1.0	1.7	24.1
	Bottom	1.8	1.5	3.1	73.3
Jun 13, 74	Surface	1.5	1.9	4.7	134.1
	Mid-Depth	1.7	3.0	4.8	112.8
	Bottom	1.2	1.2	4.4	141.9
Sep 18, 74	Surface	1.4	1.3	4.0	107.6
	Mid-Depth	1.3	1.1	2.4	34.9
	Bottom	1.6	1.3	2.3	38.3
Station 10B:					
Dec 13, 73	Surface	1.8	1.7	2.2	35.2
	Mid-Depth	1.8	1.5	1.2	7.7
	Bottom	2.0	1.6	1.0	4.8
Mar 20, 74	Surface	1.3	1.0	1.5	14.2
	Mid-Depth	1.4	1.1	1.4	10.7
	Bottom	1.7	1.5	1.8	21.2
Jun 13, 74	Surface	1.2	0.9	3.3	86.7
	Mid-Depth	1.5	1.2	1.4	10.2
	Bottom	1.2	0.9	2.1	28.1
Sep 13, 74	Surface	1.4	1.2	2.7	56.0
	Mid-Depth	1.5	1.2	1.7	16.6
	Bottom	1.6	1.7	2.1	23.2

TABLE 4-1. STATISTICAL PROPERTIES OF THE NUMBER-SIZE DISTRIBUTIONS OF THE SUSPENDED PARTICLE POPULATION OF THE UPPER CHESAPEAKE BAY (Continued)

Date	Depth	Mean (μ)	Standard Deviation (μ)	Skewness	Kurtosis
Station 11A:					
Mar 20, 74	Surface	1.4	1.0	1.1	7.0
	Mid-Depth	1.3	1.0	1.6	14.8
	Bottom	1.4	1.2	1.9	21.9
Jun 13, 74	Surface	1.4	1.1	1.7	17.1
	Mid-Depth	1.3	1.1	2.1	27.5
	Bottom	1.2	0.9	2.1	34.6
Station 11B:					
Dec 12, 73	Surface	1.8	1.3	1.1	7.8
	Mid-Depth	1.5	1.3	1.5	13.0
	Bottom	2.0	1.9	1.6	14.6
Mar 20, 74	Surface	1.2	1.0	1.8	19.3
	Mid-Depth	1.3	0.9	1.5	11.3
	Bottom	1.5	1.3	1.8	21.9
Sep 18, 74	Surface	1.4	1.5	5.1	160.5
	Mid-Depth	1.3	1.0	1.6	15.4
	Bottom	1.5	1.0	1.8	24.5

A histogram of the mean sizes, D_m, of the 140 samples analyzed for this study is plotted in Figure 39. This figure also includes a histogram for similar data from an earlier study by Schubel (1968a). In the earlier study, the 161 analyses reported by Schubel were all from the upper 40 km of the bay—upstream from Station 6. There is an apparent shift of the mean diameter toward smaller sizes in the present study relative to the data reported earlier by Schubel (1968a). One might attribute the increase in the frequency of smaller mean diameters in the present study to the influence of more seaward samples—samples which were not obtained in the earlier study. While this explanation is geologically appealing, it is not supported by the observations. No clearly defined longitudinal gradient of mean particle size was indicated by either study.

The mean size, $\overline{D}_m$, is relatively constant both temporally and spatially, ranging from 1.2 to 2.6 μ, and with 65 percent of the samples having a $\overline{D}_m$ between 1.2 to 1.6 μ. There is generally an increase in mean size near the bottom, which results from the resuspension of coarser bottom sediments. The uniformity of the number-size distribution of the suspended particles is due to the large numbers of very small particles that are ubiquitous throughout the upper bay.

There is a tendency for the number-weighted mean equivalent projected diameter, $\overline{D}_m$, to shift toward smaller values in late winter and early spring when the input of fluvial sediment is high. This shift was also reported in an earlier study by Schubel (1968a). The volume-weighted mean Stokes' diameter, however, tends to shift toward larger sizes during this period.

The skewness and kurtosis are of unknown significance in samples of very fine-grained suspended sediment. Because they are defined in terms of higher moments, they are, of course, much less stable than the mean and standard deviation. The standard deviation is a measure of the sorting of the sample and is expressed in microns. All samples were well-sorted, but the near-bottom samples tended to be somewhat less well-sorted because of the period resuspension and deposition of bottom sediments.

Histograms of the modal size class of each sample for each of the four sampling periods are plotted in Figure 4-40. A cumulative histogram for all samples is plotted in Figure 4-41. The most significant features of the suspended particle population revealed by these figures are: (1) the uniformity of the suspended particle population (particularly away from the bottom) in both time and space and (2) the preponderance of very fine particles. The upper limits of the various size classes are:

Class	Upper Limit (μ)
1	0.74
2	0.90
3	1.09
4	1.32
5	1.61
6	1.96

The number-size distribution is, of course, dominated by the more frequently occurring very small particles. Moreover, it is relatively insensitive to the much rarer large particles which, although infrequent, dominate the volume-size distribution of the suspended particle distribution.

Volume-Size Distributions - The volume-size distributions of the suspended particles were calculated from the experimentally-determined number-size distributions by making the conventional assumption that each particle population was made up of a polydisperse system of spheres. The assumption is not true, of course, but no shape factors have been determined for fine-grained natural sediments in Chesapeake Bay or elsewhere in the natural environment. Let's briefly examine some of the implications of this assumption.

When one transforms equivalent projected diameters, D_m, into equivalent volume diameters, D_v, the equivalent volume diameter is defined as the diameter of a sphere with the same *volume* as the particle in question. If the volume diameters obtained by this transformation are to provide a useful measure of the true volume diameters, either the particles should be approximately spherical, or shape factors should be employed.

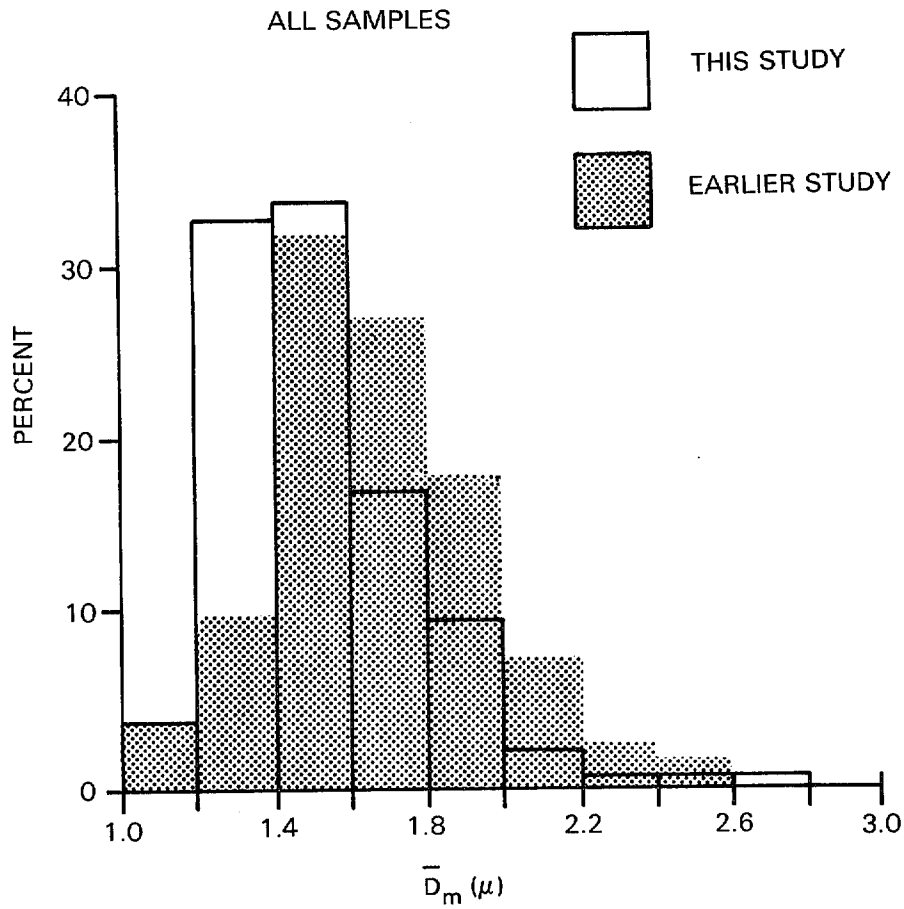

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Figure 4-39. Histograms of the Mean Sizes of All Samples. (The samples include all from the Upper Bay Survey and those of an earlier study (Schubel, 1968a) which dealt only with the upper 30 km of the bay.)

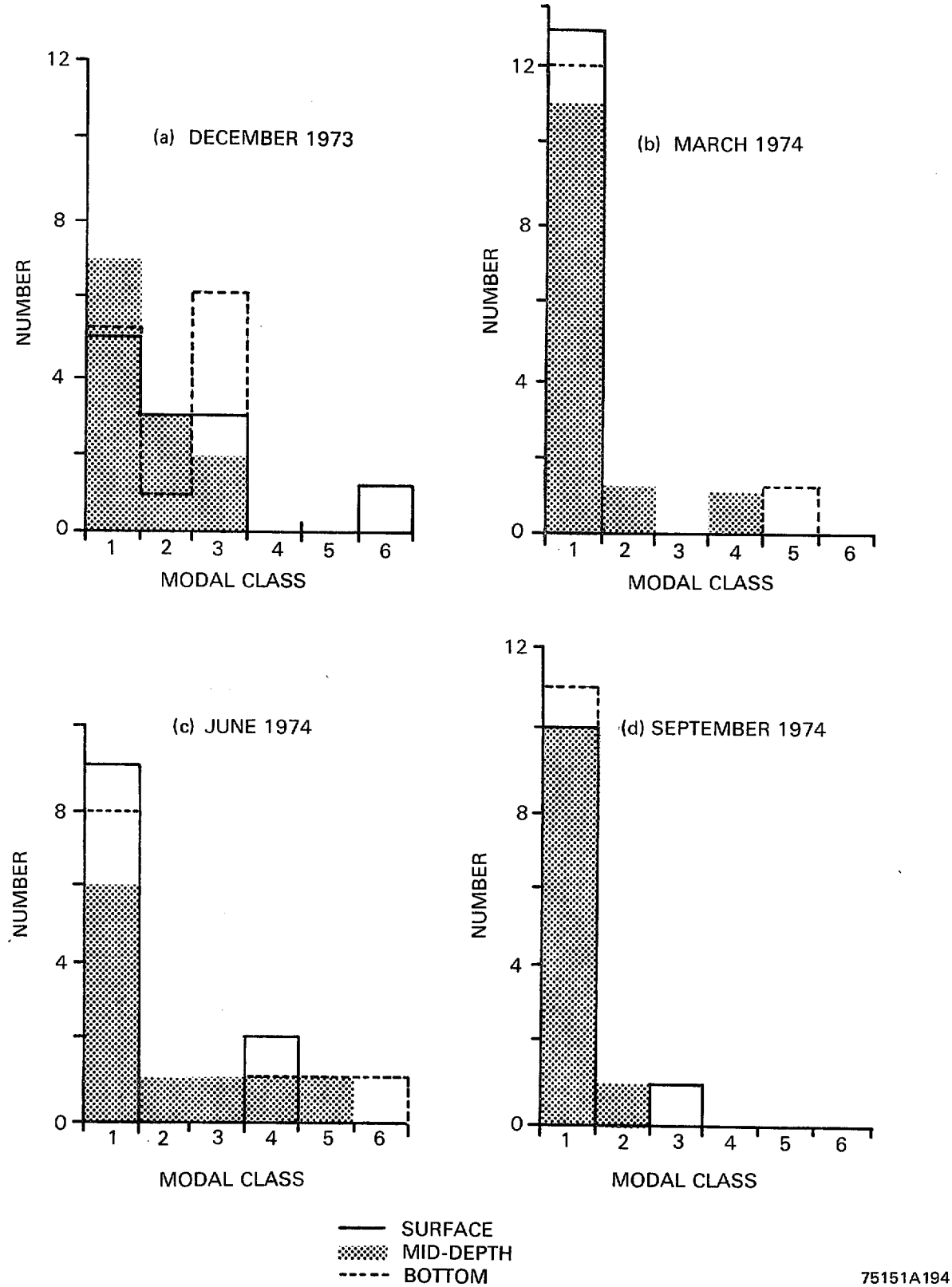

Figure 4-40. Histograms of the Modal Class of Equivalent Projected Diameters (for samples from the surface, mid-depth, and one meter above the bottom).

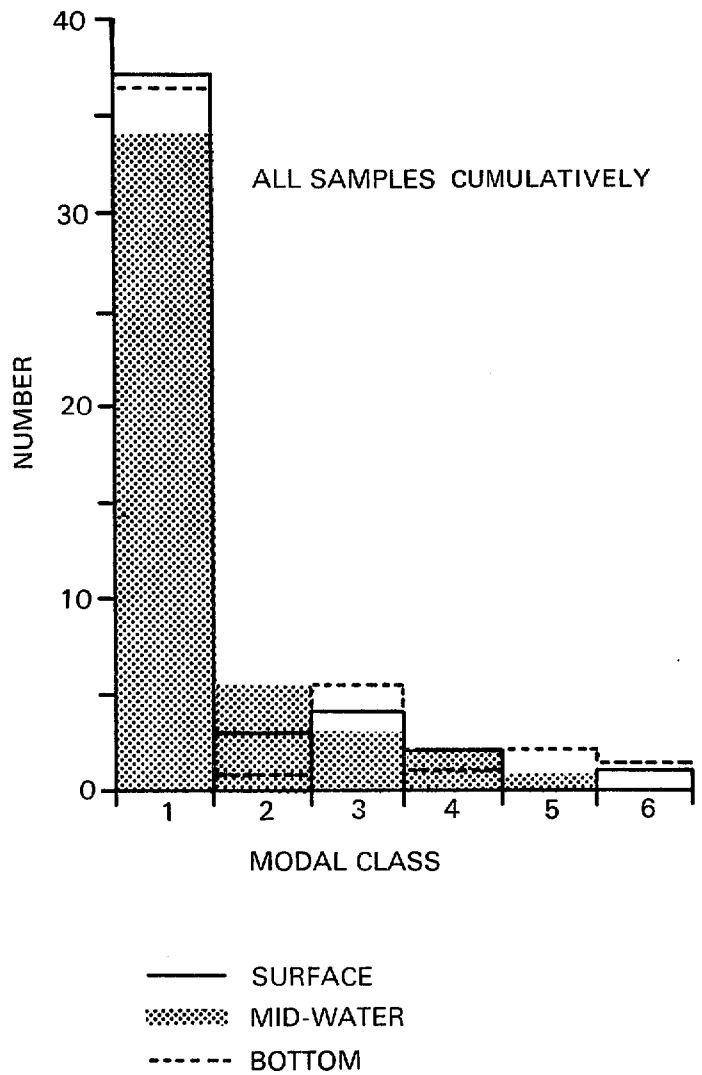

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Figure 4-41. Histogram of the Modal Class of Equivalent Projected Diameters (of all samples cumulatively from the surface, mid-depth, and one meter above the bottom) During December 1973, and During March, June, and September 1974

The bay's suspended particles have a broad spectrum of shapes ranging from spheres, through rods, to flakes. It would require an inordinate amount of work to determine shape factors for these particles, and these factors would probably change with time and space. Since no shape factors have been determined to characterize the bay's suspended particles, the volume-size distributions should be interpreted with prudence. During filtration the particles settle with their largest surfaces in the plane of the filter, and some composite particles may flatten when they contact the filter. Both of these factors result in an overestimate of the true volume diameters when the equivalent projected diameters, D_m, are cubed to obtain the volume-size distributions. In summary, the volume transformations result in an overestimate of the true volume diameters and of the statistics associated with the volume-size distributions.

An additional factor to consider is the distorting effect that a few large particles can have. Particles with equivalent projected diameters greater than 10μ are relatively rare. However, a single particle with a D_m of 20μ has a volume equivalent to that of 8,000 particles with equivalent projected diameters of 1 μ — assuming, as we have, that both have the same shape. For this reason, and those described previously, the volume-size statistics are much less stable than the number-size statistics.

All volume-size transformations are included in the Upper Bay Survey Data Base (Volume III) and are not included in this chapter. The volume-weighted mean diameter, $\overline{D}_v$, ranged from about 3 to 40μ. In more than 70 percent of the samples, $\overline{D}_v$, fell between 4 and 12μ. In more than 57 percent of the samples, $\overline{D}_v$, fell between 4 and 8μ.

Volume-Settling Velocity Distributions - Over the past ten years a relatively large number of determinations have been made of the volume-settling velocity (size) distributions of the particles suspended in the waters of the upper reaches of Chesapeake Bay. The measurements were made with a Mine Safety Appliance Particle Size Analyzer, and have been described in some detail by Schubel (1968a, 1969, 1971b). On the basis of: (1) the results for the number-size distributions and (2) preliminary tests made with a Cahn Electrobalance Sedimentation Chamber, it was determined that further tests would not increase the precision and accuracy of our estimates of the mean particle settling velocities required for the numerical model.

Results of selected analyses run previously on samples from the upper Chesapeake Bay are summarized in Table 4-2.

At nearly all stations for which samples have been analyzed, there was a displacement of the volume-settling velocity distribution curve toward larger sizes with increasing depth. This characteristic increase of the mean Stokes' diameter, $\overline{D}_s$, was almost always accompanied by an increase in the standard deviation. Schubel (1969) demonstrated that samples of suspended sediment collected from the surface waters of the upper 30 km of the bay had a range of $\overline{D}_s$ from 2.3 to 6.0μ, and in more than 75 percent of the samples it was between 2.3 and 4.0μ. At mid-depth $\overline{D}_s$ ranged from 3.4 to 6.8μ, and in over 75 percent of the samples it fell between 3.4 and 6.0μ. In the samples from one meter above the bottom, $\overline{D}_s$ ranged from 4.2 to 12.2μ and was between 4.2 and 8.0μ in more than 75 percent of the samples Schubel (1969) reported. The mean, the standard deviation, and the range of the mean all increased with depth. Schubel (1969) attributed these increases to the periodic resuspension of coarser bottom sediment by tidal scour. Later observations (Schubel, 1971) supported this conclusion.

4.2.5.3 Discussion

The size distributions of suspended sediment in the upper Chesapeake Bay are determined by a combination of physical and biological processes. There is no convincing evidence that chemical flocculation is an important process in this environment. In the upper 30 km of the bay (upstream from Tolchester) in the region characterized by a turbidity maximum, the size distributions are determined primarily by physical procees. They are the periodic resuspension of bottom sediments by tidal scour and the net non-tidal estuarine circulation (Schubel, 1972a). Biological agglomeration of fine particles by filter-feeding zooplankton also appears to be important in producing many of the larger suspended particles, and in the initial deposition of fine sediment in this part of the bay (Schubel and Kana, 1972). The formation of the turbidity maximum, discussed elsewhere in this report and previously by Schubel (1968a, 1968b), will be dealt with here only to the extent necessary for a meaningful discussion of the particle size distributions.

TABLE 4-2. STATISTICAL PROPERTIES OF VOLUME-SETTLING VELOCITY DISTRIBUTIONS OF SAMPLES OF SUSPENDED SEDIMENT FROM UPPER CHESAPEAKE BAY (SCHUBEL, 1969).

Date	Depth	$\bar{D}_s^*$ (μ)	Standard Deviation (μ)	Skewness	Kurtosis
Station Susquehanna (Same as Station 1A of Upper Bay Survey):					
Jun 1, 66	Surface	3.9	6.3	2.9	44.3
	Mid-Depth	3.9	5.7	2.9	48.8
	Bottom	4.3	6.5	2.8	42.1
Station IIIC (Near Station 4B of Upper Bay Survey):					
May 31, 66	Surface	4.9	6.2	1.9	22.3
	Mid-Depth	6.3	7.7	1.4	10.9
	Bottom	6.6	8.2	1.4	11.5
Station VF (Near Station 6C of Upper Bay Survey):					
May 31, 66	Surface	3.0	6.6	3.5	55.8
	Bottom	4.5	7.1	2.2	27.0
Station Susquehanna (Same as Station 1A of Upper Bay Survey):					
Aug 9, 66	Surface	3.6	3.5	1.5	18.3
	Mid-Depth	4.4	6.7	2.4	30.4
	Bottom	6.6	6.8	1.5	16.3
Station IIIC (Near Station 4B of Upper Bay Survey):					
Aug 22, 66	Surface	2.3	3.6	2.3	28.2
	Mid-Depth	3.5	5.2	3.2	59.2
	Bottom	8.6	13.2	2.0	22.8

* D_s is the Stokes' Diameter defined previously.

Throughout the zone of the turbidity maximum there is a *natural background* of suspended sediment which increases with depth. The intensity of the natural background at any depth is relatively constant over weeks or months. This natural background consisting of very fine-grained particles having long settling times compared to their mixing time, is attributable in part directly to runoff and in part to resuspension, primary productivity, and shore erosion. The background particle population has a relatively narrow size distribution, and the temporal and spatial variability of the mean size is small (both in terms of the volume-weighted mean Stokes' diameter, $\overline{D}_s$, and the number-weighted mean equivalent projected diameter, $\overline{D}_m$). The volume-weighted mean settling velocity of the background particles of about 10^{-3} cm/sec is of the same order as the mean vertical mixing velocity (Schubel, 1968a, 1968b), thus explaining their sustained suspension.

Mixing here is taken to include both advection and turbulent diffusion. Continuity requires that the water flowing up the Chesapeake Bay in the lower layers of water be returned seaward in the upper layers, and hence there must be a vertical advection of water from the deeper layers into the surface layers. The speed, V_a, of this net vertical flow is zero at the surface and at the bottom, but it reaches a maximum speed of the order of 10^{-3} cm/sec near mid-depth. In addition, there is a vertical diffusion velocity, V_z, due to turbulence. V_z can be defined as the ration of K_z to H, where K_z is the vertical eddy diffusivity, and H is the water depth. V_z is also of the order of 10^{-3} cm/sec.

In the lower layer at stations deeper than about four meters and throughout the water column at shallower stations superimposed upon this natural background, there are semitidal fluctuations of the suspended sediment concentration which increase in magnitude near the bottom. These semitidal fluctuations are produced by tidal scour and fill. Large particles are resuspended with increasing current speed and settle out when the current begins to wane.

Serial observations of current velocity and the concentration of suspended sediment show that maximum sediment concentrations recorded near times of maximum ebb and flood velocities exceed minimum concentrations recorded shortly after slack water by as much as a factor of 20 at one meter above the bottom. These large fluctuations of the concentration of suspended sediment produce marked changes in the volume (and weight) size distributions of the suspended particle population. At one meter off the bottom, we have recorded variations in the volume-weighted mean Stokes' diameter of from less than 4μ near slack water to more than 12μ on the preceding and succeeding maximum ebb and flood velocities of about two knots. The variation of the number size distribution is very much less because of the large numbers of background particles which are present at all times.

The increases of: (1) the mean Stokes' diameter, $\overline{D}_s$, (2) the standard deviation with depth at each station, and (3) the increase in the range of $\overline{D}_s$ with depth for samples collected from various stations at different times of the year all reflect the increasing effect of resuspension on the volume-size distribution of the suspended particle population as the bottom is approached.

Thus, the suspended particle population of the Chesapeake Bay's turbidity maximum is composed of two sub-populations(1) those particles which are in more or less continual suspension throughout the water column and (2) those particles which are alternately suspended and deposited. Throughout the year, the background subpopulation is characterized by a unform and narrow size distribution, exept for the period of the spring freshet when the hydrographic conditions are markedly changed.

During the spring freshet when most of the year's supply of new fluvial sediment is introduced (Schubel, 1968c), the increased competency of the rivers produces both a displacement of the volume-size distribution toward larger sizes and an increase in the dispersion of the volume-distribution compared to periods of moderate and low flow. The disperison of the number-size distribution of the background particles also is increased during the spring freshet, but the mean is shifted toward smaller values because of the marked increase in the relative frequency of the very fine suspended and colloidal particles.

These changes of the particle size distributions do not persist for more than a few weeks. Most of the large particles discharged during the period of high river flow are deposited within the zone of the turbidity maximum, and the very fine particles are gradually purged from the upper bay by the net non-tidal seaward flow of the upper layer.

Where tidal resuspension and deposition produces a marked change in the concentration of total suspended solids, there is a concomitant variation in the volume-settling velocity (size) distribution of the suspended particles. At two meters above the bottom in the upper reaches of the Chesapeake Bay, the concentration of suspended sediment may vary by as much as a factor of five. Serial observations of the volume-size distribution show that the distribution is successively displaced toward larger sizes with increasing ebb and flood current speeds, and then is displaced back to its background position as currents wane. The volume-weighted mean Stokes' diameter, the standard deviation, and the concentration of suspended sediment attain their maximum values near times of maximum ebb and flood current speeds, and their minimum values are attained near slack water. The mean Stokes' diameter, $\overline{D}_s$, may range from less than 3μ to more than 10μ.

Nearer the bottom—the sediment source—the same pattern is observed, but the variations in both the concentration and the particle size distribution are greater. At 0.5 m above the bottom, the concentration may vary by as much as a factor of ten, and the mean Stokes' diameter may vary from about 3μ near the time of slack water to greater than 20μ near the time of maximum ebb and flood current speeds (Schubel, 1971). With increasing distance above the bottom, the fluctuations are reduced.

Seaward of the turbidity maximum, which ends at about Tolchester, the concentrations of suspended sediment decrease. The number-size distributions do not show any consistent differences between samples collected from the zone of the turbidity maximum and those collected farther seaward in the estuary. This should be expected, however, because of the large numbers of ubiquitous small particles. Tidal fluctuations in the concentration of suspended sediment and the volume-size distribution are restricted to a smaller part of the total water column, because the mean depth increases, and because the fine-grained bottom sediments are less readily resuspended than those farther upstream. This apparently is a result of the less rapid sedimentation rates, and the greater rates of reworking by benthos in the more seaward segments of the bay.

For the purposes of the numerical model described elsewhere in Volume IV, the appropriate choice of a settling velocity for the background population of suspended particles appears to fall in the range from 1×10^{-3} to 3×10^{-3} cm/sec.

4.2.6 Acknowledgements

We thank Jeffrey Boggs and Christopher Zabawa for their assistance both in the field and in the laboratory during all phases of this project. Their contributions were manifold. We are also indebted to C. Morrow for her fine laboratory work in the initial phase of the study. We thank Captains W. J. Gilbert and C. Wessels for their unfailing cooperation. Both of these men have the capacity to make sea work enjoyable even under the most trying conditions.

Contribution 223 of the Chesapeake Bay Institute of The Johns Hopkins University, and Contribution 135 of the Marine Sciences Research Center of the State University of New York.

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CHAPTER 5

HYDROLOGY AND METEOROLOGY

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ABSTRACT

Chlorinated hydrocarbon pesticides and polychlorinated biphenyls were found in all major phases of the hydrologic cycle—in the atmosphere, precipitation, storm water runoff, ground water flow, and receiving water bodies. Such significant findings were revealed in this study as the presence of 9 ng/m^3 of PCB in the air, 580 parts per trillion (ppt) of PCB in storm water, 283 ppt of toxaphene, and 180 ppt of chlordane in rain water. Hydrological and meteorological parameters of concern also are presented and discussed for interpretation and correlation purposes.

It is concluded that air and water movements are the major transport media for the chlorinated hydrocarbons. Rates of transport of these compounds in the atmosphere in precipitation and aerial fallout, and in stream flow are estimated based on some reasonable assumptions. Relatively high levels of chlorinated hydrocarbons in the storm water runoff indicate the need for a comprehensive study of the storm water inflow to the Chesapeake Bay and its water quality.

TABLE OF CONTENTS

Subject		Page
5.	HYDROLOGY AND METEOROLOGY	5-1
5.1	Introduction	5-1
5.2	Data Acquisition Techniques	5-4
5.2.1	Meteorological Data	5-4
5.2.2	Physical Parameter Cruises	5-4
5.2.3	Multidisciplinary Cruises	5-4
5.2.4	Tidal Data Collections	5-4
5.2.5	Stream Flows	5-8
5.2.6	Chlorinated Hydrocarbons in Air-Dust, Rain Water, Storm Water and Ground Water	5-8
5.3	Results and Discussion	5-12
5.3.1	Chlorinated Hydrocarbons in Air-Dust	5-12
5.3.2	Chlorinated Hydrocarbons in Rain Water	5-13
5.3.3	Chlorinated Hydrocarbons in Storm Water	5-13
5.3.4	Chlorinated Hydrocarbons in Ground Water	5-13
5.3.5	Climatological Variations	5-18
5.3.6	Variations of Water Temperature, Salinity, and Suspended Sediment	5-18
5.3.7	Fresh Water Inputs and Tidal Variations	5-18
5.4	Correlation Analyses and Discussion	5-30
5.4.1	Rate of Transport of Chlorinated Hydrocarbons in the Atmosphere	5-30
5.4.2	Chlorinated Hydrocarbons Return to Earth	5-30
5.4.3	Chlorinated Hydrocarbons in Storm Water Runoff and Precipitation	5-32
5.4.4	Spring Freshet: Inputs of Sediments and Chlorinated Hydrocarbons	5-32
5.5	Conclusions	5-36
5.6	Acknowledgements	5-37
5.7	References	5-37

LIST OF FIGURES

Figure		Page
5-1	Flow Chart Showing the Kinetics of Pesticides	5-2
5-2	The General Hydrologic Cycle and its Long-Time Averages	5-3
5-3	Station Location Map for the Upper Chesapeake Bay	5-6
5-4	Station Location Map for the Chester River	5-7
5-5	Locations of Morrell Park, Sollers Point, and Fort Smallwood	5-9
5-6	Air-Dust Sampling Device	5-10
5-7	Installation of Air Sampling System at Sollers Point	5-10
5-8	Installation of Rain Collector at Fort Smallwood Park	5-11
5-9	Storm Water Manhole on the DeSoto Road, Morrell Park	5-11
5-10	Variations of Water Temperature, Salinity, Sigma-t and Suspended Sediment at Stations 01A and 04B	5-23
5-11	Variations of Water Temperature, Salinity, Sigma-t and Suspended Sediment at Stations 07A and 11B	5-24
5-12	Estimated Monthly Streamflow Entering Chesapeake Bay and Annual Mean Flow	5-25
5-13	Cumulative Inflow to Chesapeake Bay, 1974	5-26
5-14	Daily Mean Discharges for the Susquehanna River at Conowingo, Maryland	5-27
5-15	Actual Water Level Variations at Havre de Grace and Matapeake, April, 1974	5-28
5-16	Actual Water Level Variations at Havre de Grace and Matapeake, October, 1974	5-28
5-17	Wind Rose for July 1974 at Baltimore Washington International Airport	5-29
5-18	Monthly Resultant Wind Speed and Direction at BWI Airport for 1974	5-31
5-19	Precipitation and Concentrations of Chlorinated Hydrocarbons in Storm Water	5-33
5-20	Hourly Precipitations at Baltimore March, 1974	5-34
5-21	Estimated Monthly Inputs of Suspended Sediment from Susquehanna River	5-35

LIST OF TABLES

Table		Page
5-1	Summary of Hydrological and Meteorological Data, Upper Bay Survey	5-5
5-2	Chlorinated Hydrocarbons on Gelman Filter and Polyurethane Foam Plugs	5-14
5-3	Chlorinated Hydrocarbons in Air-Dust Samples	5-14
5-4	Chlorinated Hydrocarbons and Suspended Particulate Matter in Air-Dust Samples	5-15
5-5	Chlorinated Hydrocarbons in Rain Water	5-16
5-6	Chlorinated Hydrocarbons in Storm Water Samples	5-17
5-7	Chlorinated Hydrocarbons in Ground Water Samples	5-17
5-8	Monthly Averages and Departures From Normal for Air Temperature, etc.	5-19
5-9	Water Temperature, Salinity, and Concentration of Suspended Sediment at Station 1A	5-20
5-10	Water Temperature, Salinity, and Concentration of Suspended Sediment at Station 4B	5-21
5-11	Water Temperature, Salinity, and Concentration of Suspended Sediment at Station 11B	5-22

5. HYDROLOGY AND METROLOGY

5.1 Introduction

Hydrological and meteorological aspects of the study were focused on the assessment of the sources and transport mechanisms of the chlorinated hydrocarbon pesticides and polychlorinated biphenyls (PCB's) in the upper Chesapeake Bay. Figure 5-1 is a flow diagram showing the kinetics of pesticides from the point of application through various media and finally entering human beings (Tzou, 1972). Routes and modes of transport for PCB's are essentially similar to those for pesticides (Figure 5-1) after they enter the ecological system.

In general, fluid movements—air and water—are responsible for the transport of chlorinated hydrocarbon residues. The occurrence of residues in air and water at points far from sites of application can be illustrated from previous studies. In the latter half of 1964, DDT in dust over Pittsburgh averaged 0.24 ppt. Cohen and Pinkerton (1966) collected dust-rain which was brought to Ohio by a dust storm originating in the Texas-Oklahoma-New Mexico area. Scientists from Michigan State University have found that the DDT concentration was highest in rivers having runoff originating primarily from city discharges rather than from agricultural areas. Another study in California indicated that about two tons of DDT enters San Francisco Bay every year from the Sacramento and San Joaquin Rivers. The Westinghouse Chester River Study showed that 28 grams of PCB, three grams of total DDT, and one gram of technical chlordane moved into Chester River each day from the Chesapeake Bay during the early spring freshet period, and only a small fraction of these compounds returned to the bay in late spring. (Tzou, 1972).

In any ecological system study, the hydrologic cycle (Figure 5-2) always plays an important role as a transport media for pollutants. Evaporation-transpiration and precipitation-runoff are the main phases of the cycle (Butler, 1957). Most of the chlorinated hydrocarbons originate from agricultural, municipal, and industrial areas. The major mechanisms for carrying these compounds from the land areas into the estuarine environments are wind drift, precipitation, surface runoff, storm sewer flow, and ground water flow. Vaporized chlorinated hydrocarbons in the air will be partially adsorbed on particulates and transported in the form of vapors and suspended particulates. Chlorinated hydrocarbon residues in

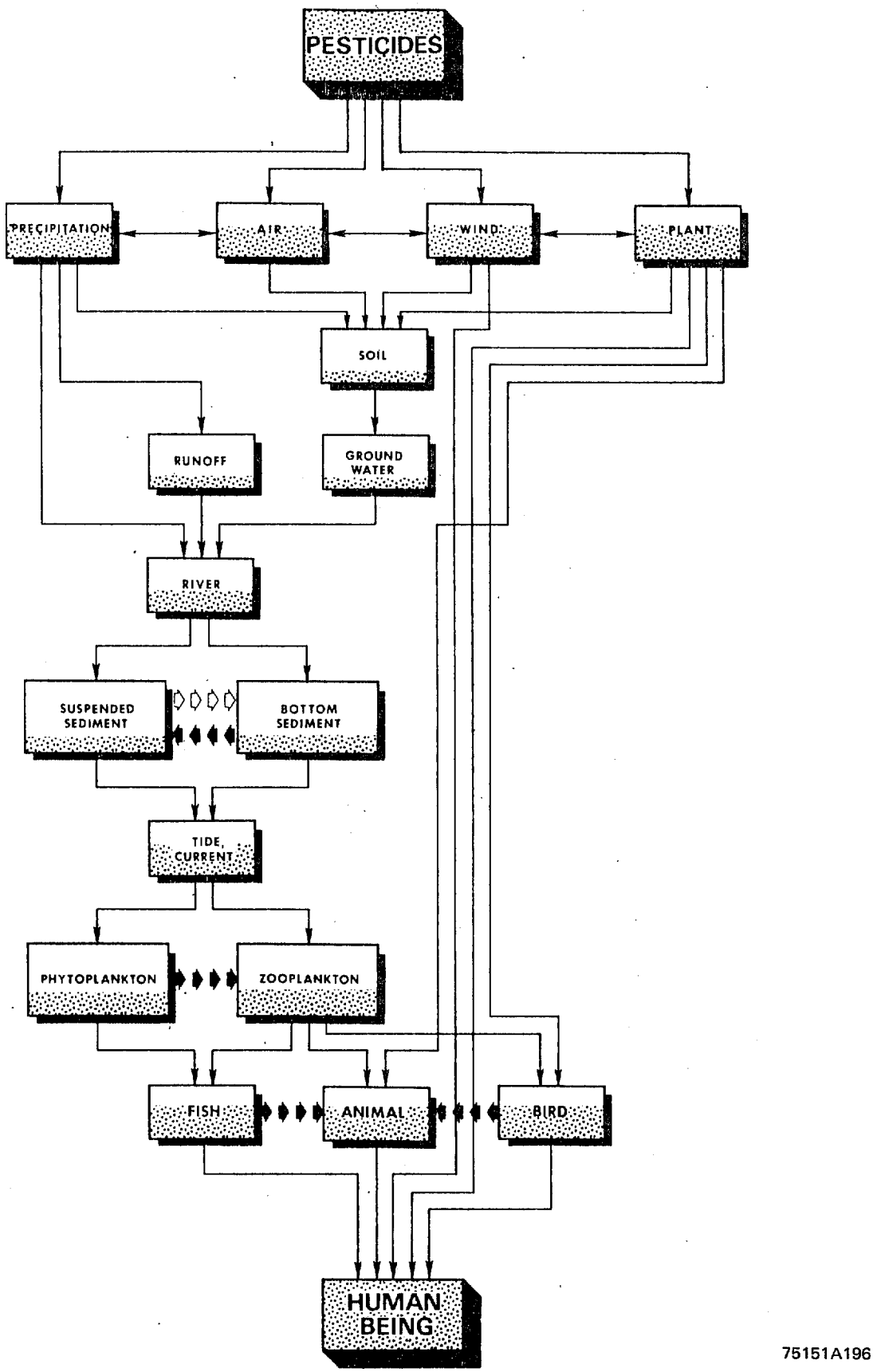
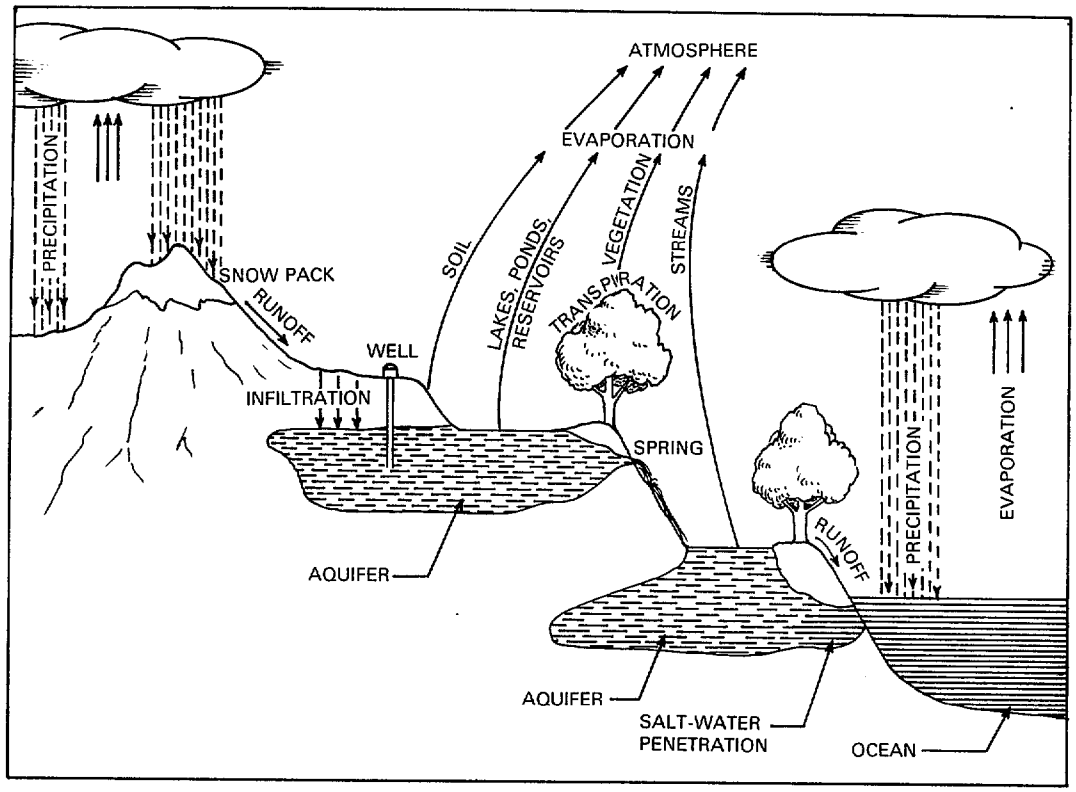


Figure 5-1. Flow Chart Showing the Kinetics of Pesticides (Tzou, 1972)



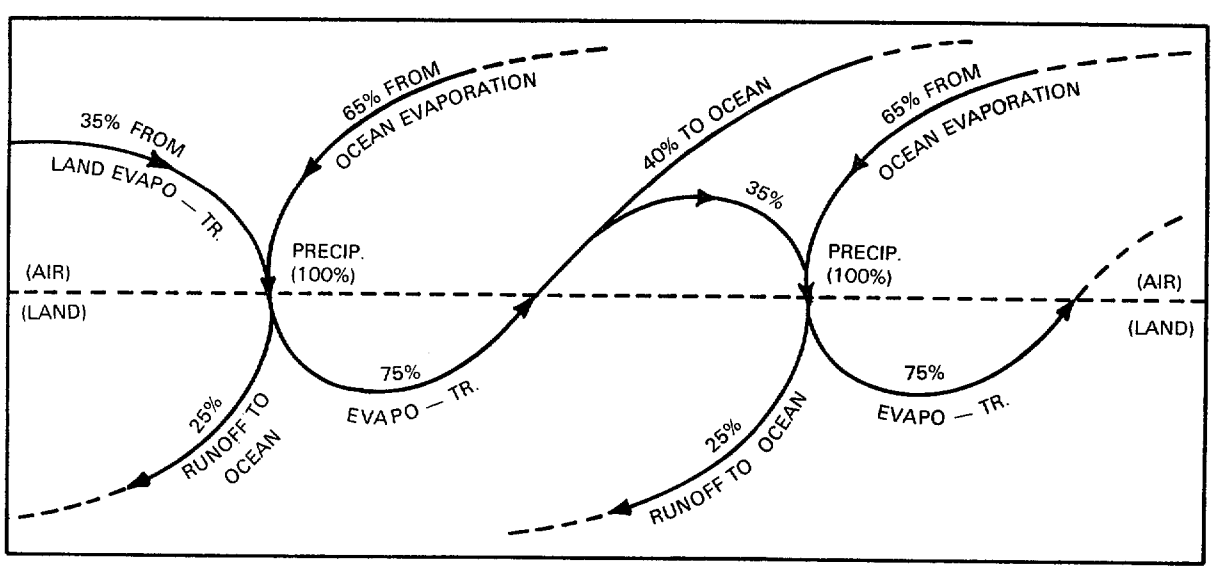


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Figure 5-2. The General Hydrologic Cycle (above) and its Long-Time Averages for the USA (below) (Butler, 1957 after the Rand Corp.)

water bodies tend to adsorb to the suspended sediment particles. Because of their abundance and large surface area, the smallest particles seem to carry the greatest residue burden. Hence, the rates of transport of chlorinated hydrocarbons can be evaluated from analyzing the transport of suspended sediment in the water system.

Descriptions of the data-gathering techniques, sample analyses, discussions of the results, and conclusions are presented in the subsequent sections of this chapter. Supportive graphs and tabulations of data from hydrological and meteorological studies can be found in Volume III, Appendix A.

5.2 Data Acquisition Techniques

Collection of hydrological and meteorological data is critical to the understanding of the transport mechanism of chlorinated hydrocarbons in the environment. In this study, data have been collected by a variety of methods from various sources. Table 5-1 summarizes the hydrological and meteorological data, and Figure 5-3 shows the locations of the sampling stations.

5.2.1 Meteorological Data

The National Weather Service of NOAA at Baltimore Washington International Airport provided the meteorological data for the period from December 1973 through December 1974. Parameters included hourly variations of air temperature, barometric pressure, precipitation, wind speed, and wind direction.

5.2.2 Physical Parameter Cruises

In support of the development of a mathematical model, Westinghouse participated in two field sampling programs during The Chesapeake Bay Institute's two 10-day cruises. The first cruise was conducted in the period from April 17 through April 20, 1974. Measured parameters included water temperature, conductivity, and suspended sediments. Westinghouse's *R/V NORTH STAR* occupied Station T3 on April 17 and 18 then moved to Station S3 on April 19 and 20 (Figure 5-3).

The second physical parameter cruise was accomplished in the period from October 8 through 11, 1974. The Westinghouse vessel occupied Station SS4 on October 8, from 6:00 AM to 6:00 PM, measuring parameters including water temperature, salinity, and suspended sediment (Figure 5-4). On October 9, it conducted the same operations except that no suspended sediment samples were taken. On October 10 and 11, it conducted two slack runs each day from Station C1 through Station C10, measuring temperature and salinity in the water column.

5.2.3 Multidisciplinary Cruises

In order to provide synoptic measurements in the collection of field data for different disciplines, six multidisciplinary cruises were conducted by a joint team of personnel from Chesapeake Bay Institute, University of Maryland, and Westinghouse Oceanic Division. Hydrological parameters included in the missions were water temperature, salinity, and suspended sediments in the water column. The six cruises were performed December 12 through 14, 1973, January 23 through 25, March 19 through 21, May 3 through 5, July 31 through August 2, and September 18 through 20, 1974.

5.2.4 Tidal Data Collections

The Oceanographic Division of NOAA furnished the latest available tidal data at three gauging stations—Havre de Grace, Baltimore, and Matapeake for the period from December 1973 through December 1974.

TABLE 5-1. SUMMARY OF HYDROLOGICAL AND METEOROLOGICAL DATA, UPPER BAY SURVEY, DEC. 1973 – DEC. 1974

	1973	1974											
	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
I. Meteorological Data	*	*	*	*	*	*	*	*	*	*	*	*	*
II. Physical Parameter Cruises					*						*		
III. Multidisciplinary Cruises	*	*		*		*		*		*			
IV. Tide Data:													
i. Havre de Grace	*	*	*	*	*	*	*	*	*	*	*	*	*
ii. Baltimore			*	*	*	*	*	*					*
iii. Matapeake			*		*	*	*	*	*	*	*		*
V. Stream Flows													
i. Susquehanna River	*	*	*	*	*	*	*	*	*	*	*	*	*
ii. Patapsco River	*	*	*	*	*	*	*	*	*	*	*	*	*
VI. Chlorinated Hydrocarbons													
i. Air-Dust								*	*		*		
ii. Rain Water								*	*	*	*		
iii. Storm Water				*		*				*	*	*	
iv. Ground Water						*		*		*	*	*	

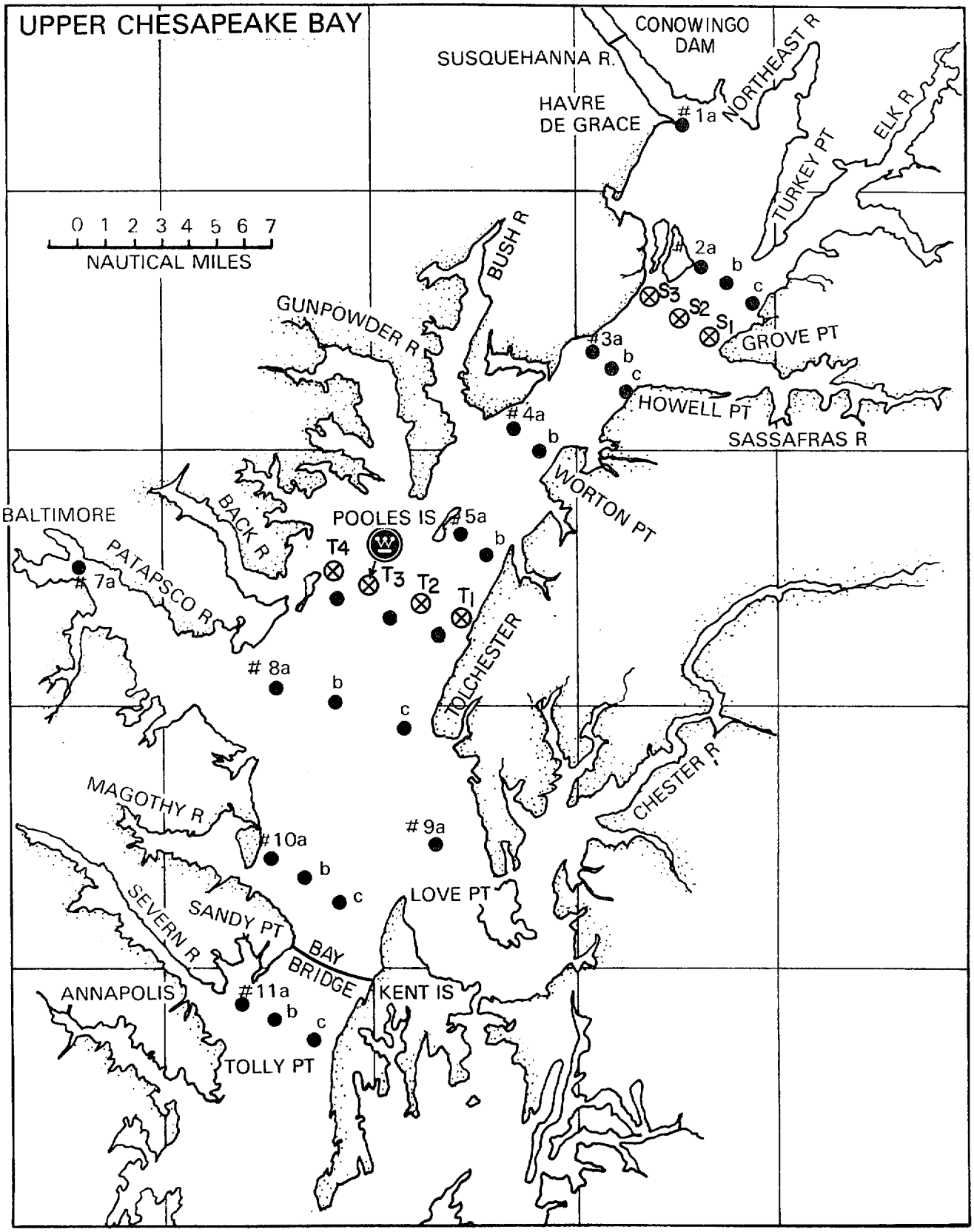

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Figure 5-3. Station Location Map for the Upper Chesapeake Bay

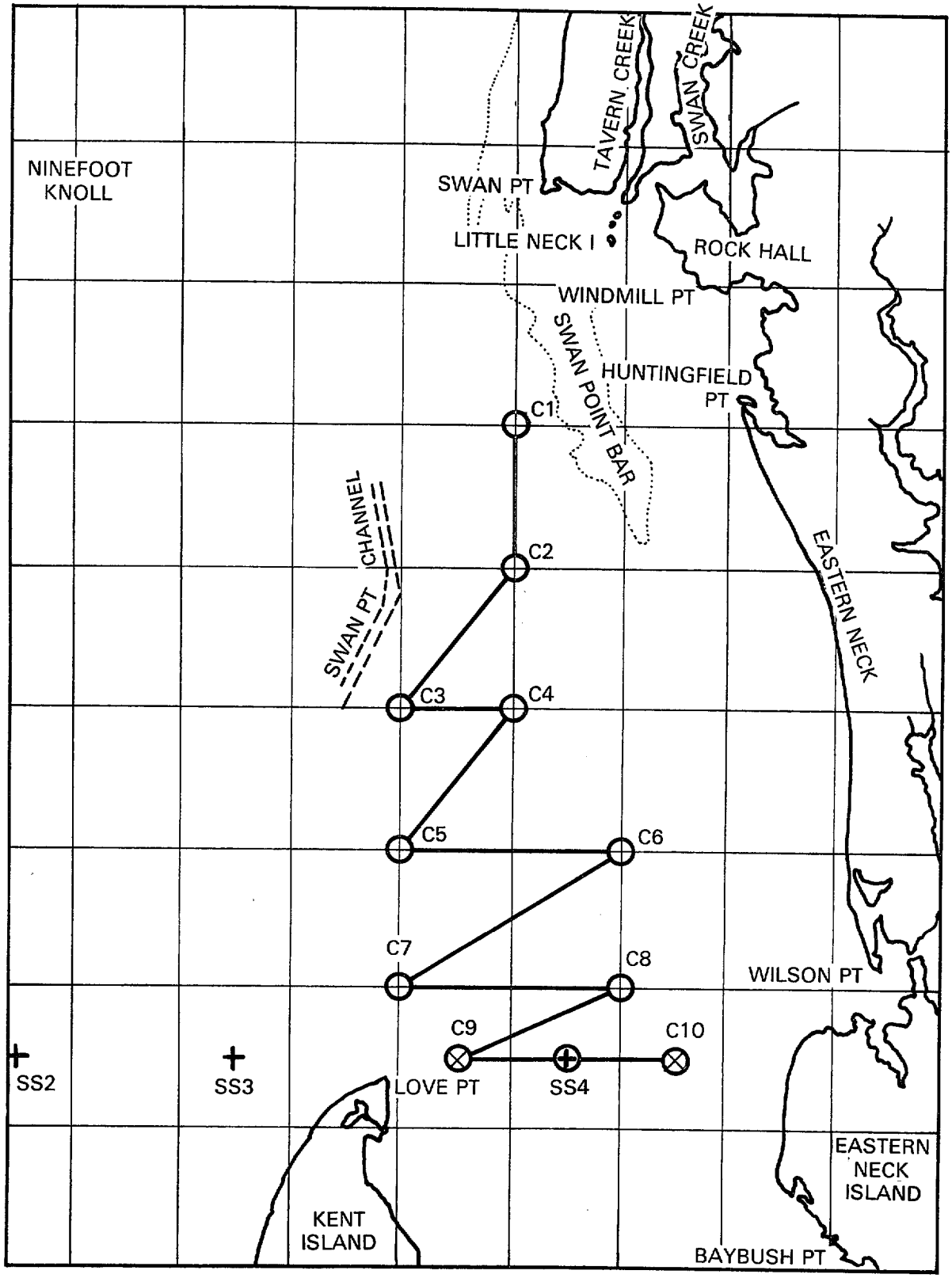

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Figure 5-4. Station Location Map for the Chester River

5.2.5 Stream Flows

The major fresh water inflow to the upper Chesapeake Bay is from the Susquehanna River. The Water Resources Division, U. S. Geological Survey provided the streamflow data for the Susquehanna river at Conowingo Dam and for the Patapsco River at Hollofield, Maryland for the period from December 1973 through December 1974.

5.2.6 Chlorinated Hydrocarbons in Air-Dust, Rain Water, Storm Water and Ground Water

Figure 5-5 shows the locations of Morrell Park, Sollers Point, and Fort Smallwood at which we collected storm water, air-dust, rain water, and ground water samples.

Air-dust Samples—The chlorinated hydrocarbon air sampling system used in this study is essentially similar to the high-volume air collector of the University of Rhode Island (Bidleman and Olney, 1973). The initial collector is a Gelman paper filter (pore size 0.3 μm). This is backed by an aluminum cylinder ten inches long and three inches in diameter containing two 3 in. x 3 1/2 in. polyurethane foam plugs. The foam plugs are one-half inch larger than the inside diameter of the aluminum cylinder to ensure a tight fit. The outlet of the air vacuum pump (Cadillac Air Pump) is connected to a flow meter (Fisher and Porter Product) to measure the flow rate passing through the system (Figure 5-6).

Figure 5-7 shows the installation of the air sampling system at Sollers Point, a State of Maryland air quality sampling station. Six air samples were collected during the study. A 24-hour sampling period was chosen in coordination with the State's sampling program. Flow rates were recorded at the beginning and at the end of the period, assuming a linear variation during the sampling period. The total volume of air passing through the system ranged from 600 to 750 m^3/day.

Rain Samples—Two types of rain collectors were used in this study. A one-meter square box made of sheet metal was installed at Fort Smallwood Park (Figure 5-8). The maximum capacity of this collector was five gallons.

Another type of rain collector was a glass container of 5 in. x 6 in. x 11 in. (height) with a small amount of mineral oil on the bottom to prevent the evaporation of collected water. It was installed at Sollers Point station at the same site as the State's air-dust fallout collector. Samples collected from these devices represented total precipitation and fallout from the atmosphere.

Storm Sewer Samples—The storm sewer outlet near DeSoto Road in Morrell Park, designed to carry storm water only, collects the runoff from a local residential area. Six five-gallon samples were collected here and analyzed for this study (Figure 5-9).

Ground Water Samples—One of the U. S. Geological Survey observation stations, a brick-lined well at Fort Smallwood Park, was selected for sampling ground water. It has a diameter of four feet and a water level about 14 feet from the ground. Five five-gallon samples were collected here and analyzed for this study.

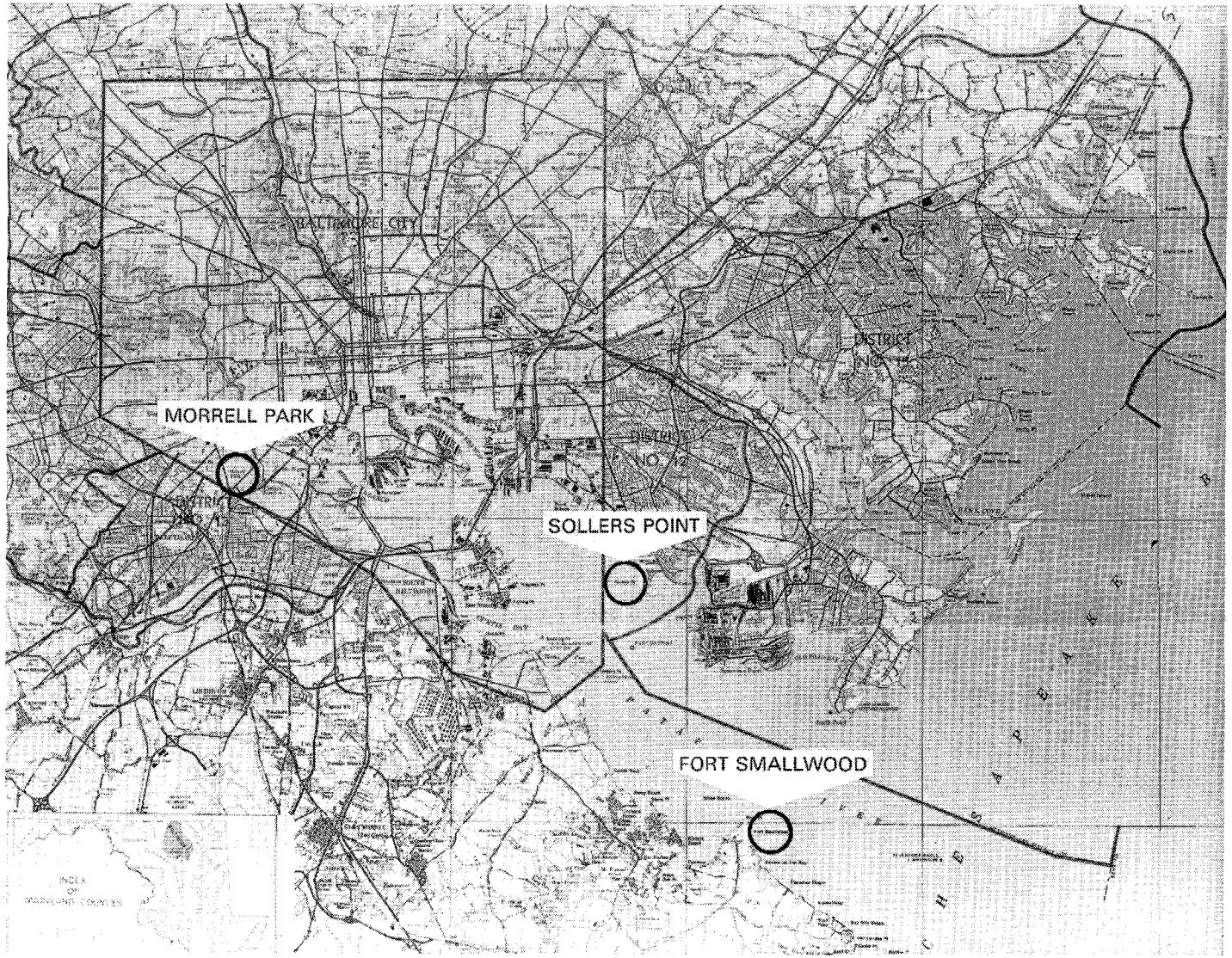

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Figure 5-5. Locations of Morrell Park, Sollers Point, and Fort Smallwood

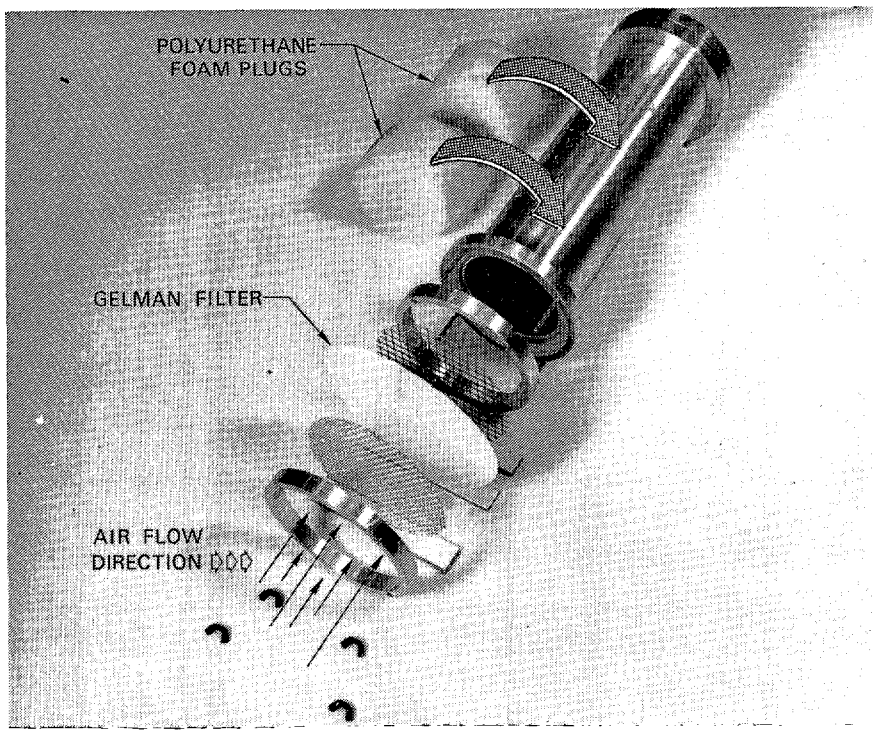

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Figure 5-6. Air-Dust Sampling Device

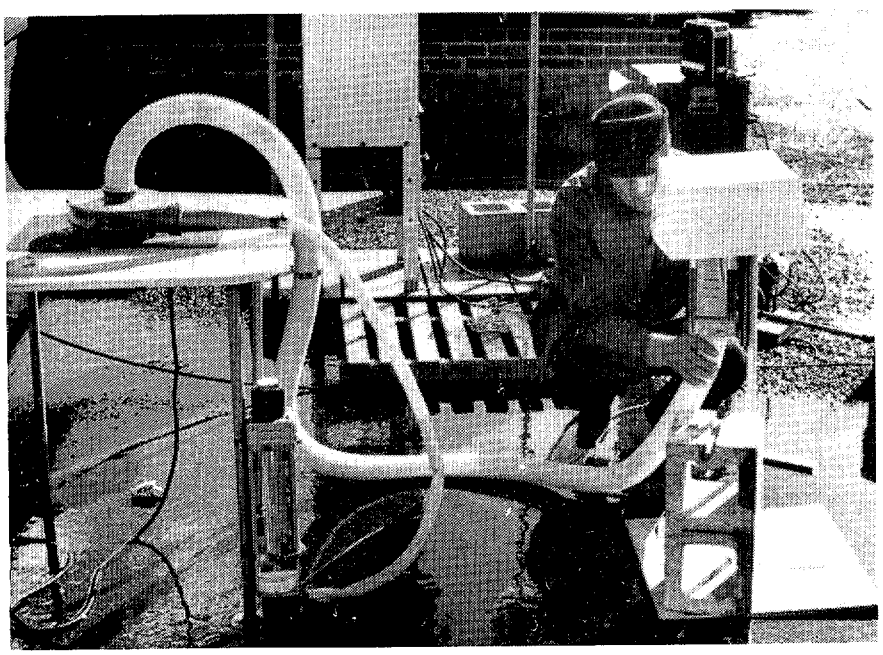

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Figure 5-7. Installation of Air Sampling System at Sollers Point

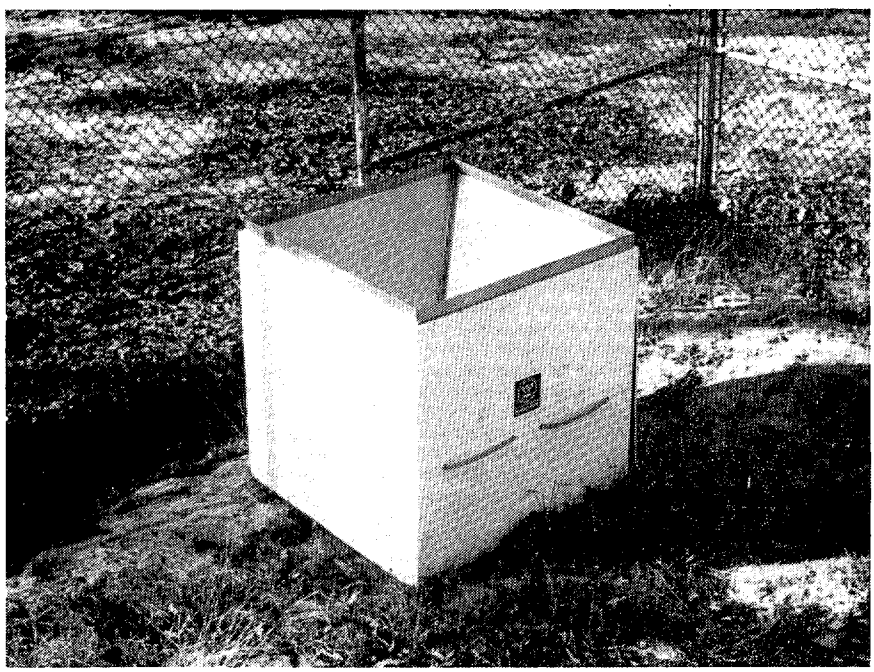

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Figure 5-8. Installation of Rain Collector at Fort Smallwood Park

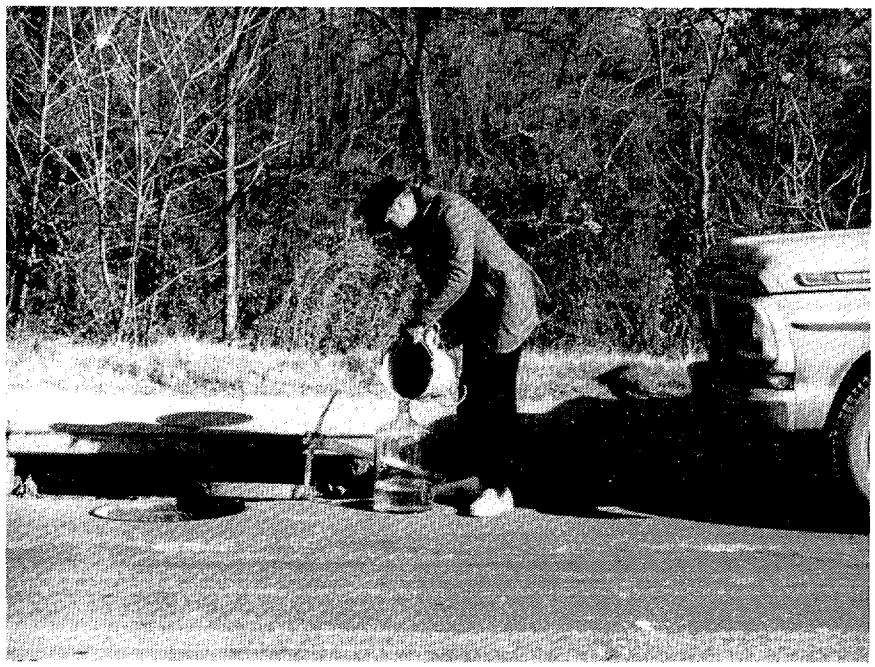

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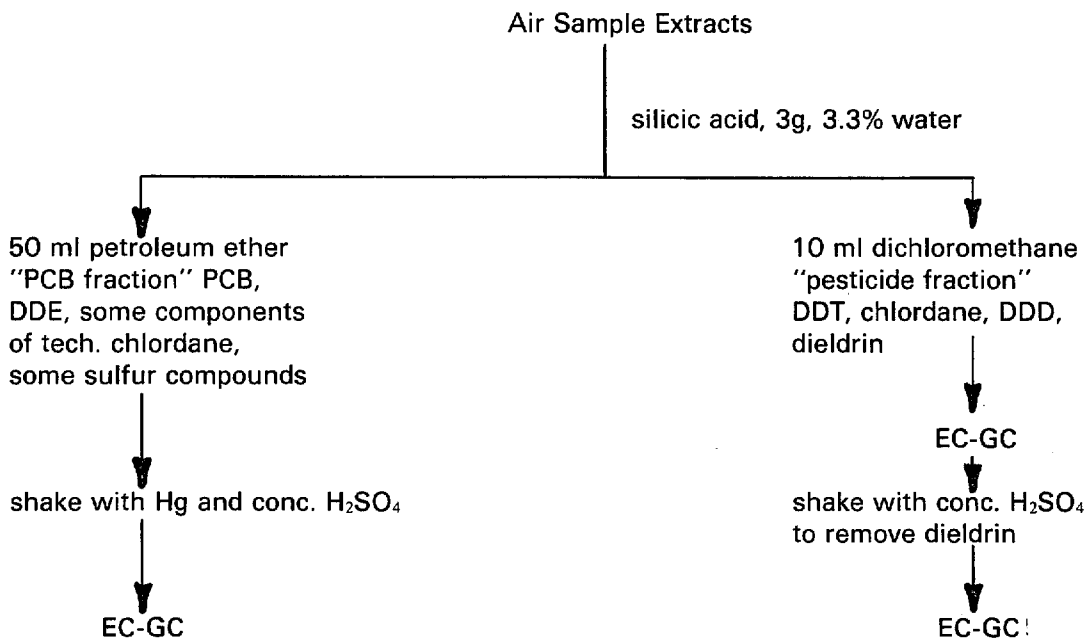
Figure 5-9. Storm Water Manhole on the DeSoto Road, Morrell Park

5.3 Results and Discussion

5.3.1 Chlorinated Hydrocarbons in Air-Dust

The atmosphere has been suggested by many researchers as a major transport route of chlorinated pesticides (including PCB's) to the ocean (Risebrough et al., 1968; Cohen et al., 1966; Nisbet et al., 1972; Bidleman et al., 1974). In this study, six air-dust samples were collected from Sollers Point station; they were extracted and analyzed at the University of Rhode Island. A flow diagram describing the analytical procedure is presented below (Bidleman, 1974):



In the beginning of the field data collection, one four-inch Gelman filter followed by two 3 in. x 3 1/2 in. diameter polyurethane foam plugs were used to trap chlorinated hydrocarbons from the air. The analytical results for the sample collected on July 22, 1974 are presented in Table 5-2.

Three principal findings resulted. First, only 9 percent of PCB and 18 percent of chlordane were found on the Gelman filter, although the filter was very dirty (estimated residue at about 55 mg of suspended particulate matter on the filter). Secondly, the Gelman filter contained almost no dieldrin nor DDT, which appeared on the first foam plug instead. Thirdly, the second foam plug contained no chlorinated hydrocarbons. Based on the experimental results of this sample, it was decided to eliminate the second foam plug in the subsequent data collection.

TABLE 5-2. CHLORINATED HYDROCARBONS ON GELMAN FILTER AND POLYURETHANE FOAM PLUGS

Sollers Point, July 22, 1974, Air Volume = 586 m^3 (24 hours)

	PCB (Aroclor® 1254) (ng)*	Chlordane (alpha plus gamma) (ng)	Dieldrin (ng)	DDT (ng)
Gelman Filter	342	221	– –	– –
First Plug	3,432	982	123	62
Second Plug	– –	– –	– –	– –
Total	3,774	1,203	123	62

**The abbreviation ng is nanograms.

Table 5-3 presents the total weight of chlorinated hydrocarbons in the filter and foam plug in nanograms per sample. Concentrations of chlorinated hydrocarbons and suspended particulate matter in air-dust samples are shown in Table 5-4. Among various chlorinated hydrocarbons found in the air samples, PCB (Aroclor® 1254) had the highest concentration, ranging from three to nine nanograms (ng) per cubic meter. The highest chlordane (alpha plus gamma) concentration was three nanograms per cubic meter. The DDT and dieldrin concentrations were relatively small.

In a field study conducted by EPA in 1967 and 1968 (Stanley, 1971), the maximum levels of pesticides found in air samples from Baltimore were:

p,p' - DDT - 20 ng/m^3
o,p' - DDT - 3 ng/m^3
p,p' - DDE - 2 ng/m^3
Lindane - 3 ng/m^3
Alpha - BHC - 5 ng/m^3
Gamma - BHC - 2 ng/m^3

Because of the different localities and methods of collection, it is difficult to compare the results of the U. S. Environmental Protection Agency's study and those of the present study. However, from previous work it can be concluded that the level of DDT (p,p' -) in the atmosphere has decreased in recent years.

5.3.2 Chlorinated Hydrocarbons in Rain Water

The airborne chlorinated hydrocarbons return to earth mainly through rain and, to a lesser extent, by falling dust (Matsumura, 1972). In this study, a total of eight rain samples were collected and analyzed. Concentrations of chlorinated hydrocarbons in each of the rain samples are presented in Table 5-5.

At the Fort Smallwood station, toxaphene as high as 283 ppt was detected in the sample collected from August 28 through September 4. Maximum concentrations of chlordane and benzene hexachloride (BHC) appeared in the October sample with 4 ppt for chlordane (alpha plus gamma) and 51 ppt for BHC (alpha plus gamma). The PCB's and DDT were considerably lower than other concentrations at the 0-4 ppt level.

TABLE 5-3. CHLORINATED HYDROCARBONS IN AIR-DUST SAMPLES, SOLLERS POINT, MARYLAND, 1974

Date	Air Volume (m^3)	PCB (Aroclor® 1254) (ng*)	DDT (pp'-) (ng)	Chlordane Alpha (ng)	Chlordane Gamma (ng)	Dieldrin (ng)
July 16 (24 hours)	673	2,701	110	510	491	215
July 22 (24 hours)	586	3,774	62	648	555	123
July 30–31 (48 hours)	1,448	9,425	--	1,988	1,742	--
August 21 (24 hours)	745	6,778	--	715	640	55
October 8 (24 hours)	620	1,455	9	370	365	74
October 26 (24 hours)	628	1,566	9	191	205	52

*The abbreviation ng is nanograms.

Samples collected at the Sollers Point station (Table 5-5) were quite different from those collected at the Fort Smallwood station. Higher concentrations of PCB's (130 ppt) and chlordane (180 ppt for alpha plus gamma chlordane) were detected. The difference in results for the two sampling stations is probably attributable to the locality. Fort Smallwood is a recreation park, and Sollers Point is a residential area with an industrial neighborhood, hence, closer to a variety of sources of these materials.

5.3.3 Chlorinated Hydrocarbons in Storm Water

Materials commonly on street surfaces have been found to contribute substantially to urban pollution when washed into receiving waters by storm runoff (Sartor and Boyd, 1972). A pilot study was conducted for the Upper Bay Survey in order to determine the concentrations of chlorinated hydrocarbons in a storm water system of Baltimore. Table 5-6 presents the analytical results of the six samples taken. Concentrations as high as 580 ppt for PCB and 63 ppt for chlordane were found in the sample collected on March 29, 1974.

5.3.4 Chlorinated Hydrocarbons in Ground Water

After entering the surface runoff, water-borne pollutants may infiltrate into the ground water system. Five ground water samples were collected and analyzed to compare the concentrations of chlorinated hydrocarbons in various hydrologic systems. Total PCB ranged from 1 to 26 ppt; total DDT varied from 1 to 6 ppt; highest chlordane was 2 ppt, and highest dieldrin was 3 ppt (Table 5-7).

TABLE 5-4. CONCENTRATIONS OF CHLORINATED HYDROCARBONS AND SUSPENDED PARTICULATE MATTER IN AIR-DUST SAMPLES, SOLLERS POINT, MARYLAND, 1974

Date	PCB (Aroclor 1254) (ng/m^3)*	DDT (pp'-) (ng/m^3)	Chlordane Alpha (ng/m^3)	Chlordane Gamma ng/m^3)	Dieldrin (ng/m^3)	Suspended Particulate Matter ($\mu g/m^3$)
July 16 (24 hours)	4	<1	<1	<1	<1	91
July 22 (24 hours)	6	<1	1	1	<1	94
July 30–31 (48 hours)	7	—	2	1	—	
August 21 (24 hours)	9	—	1	<1	<1	
October 8 (24 hours)	3	—	<1	<1	<1	61
October 26 (24 hours)	3	—	<1	<1	<1	

*The abbreviation ng is nanograms, and μg is micrograms.

TABLE 5-5. CONCENTRATIONS OF CHLORINATED HYDROCARBONS IN RAIN WATER

Date (Time)	Total PCB (ppt)	Total DDT (ppt)	Chlordane Alpha (ppt)	Chlordane Gamma (ppt)	Toxaphene (ppt)	Benzene Hexachloride Alpha (ppt)	Benzene Hexachloride Gamma (ppt)
Samples at Fort Smallwood, Maryland, 1974							
Jul 30 (12:00 noon) – Aug. 7 (8:00 AM)	2	2					
Aug. 28 (12:00 noon) – Sep. 4 (8:15 AM)			2	<1	283	15	2
Sep. 4 (8:15 AM) – Sep. 28 (12:00 noon)					167	4	3
Sep. 28 (12:00 noon) – Oct. 1 (8:30 AM)			<1	<1	44	11	4
Oct. 1 (8:30 AM) – Oct. 16 (10:00 AM)		4	2	2	86	37	14
Samples at Sollers Point, Maryland, 1974							
Jul. 29 (10:00 AM) Aug. 30 (10:00 AM)	130	11	23	13		18	
Aug. 30 (10:00 AM) Oct. 3 (7:30 AM)			19	15	220		
Oct. 3 (7:30 AM) Nov. 1 (8:30 AM)	120	22	94	90			

TABLE 5-6. CONCENTRATIONS OF CHLORINATED HYDROCARBONS IN STORM WATER SAMPLES, MORRELL PARK, MARYLAND, 1974

Date (Time)	Total PCB (ppt)	Total DDT (ppt)	Chlordane Alpha (ppt)	Chlordane Gamma (ppt)	Toxaphene (ppt)	Benzene Hexachloride Alpha (ppt)	Benzene Hexachloride Gamma (ppt)
Mar. 29 (8:30 AM)	27	12	9	2	--	--	--
Mar. 29 (6:00 PM)	580	5	42	21	--	--	--
May 3 (7:30 AM)	46	31	--	--	--	--	--
Sep. 4 (7:30 AM)	190	12	4	2	--	--	--
Oct. 16 (9:00 AM)	--	--	<1	<1	13	2	<1
Nov. 5 (11:00 AM)	7	7	8	5	--	--	3

TABLE 5-7. CONCENTRATIONS OF CHLORINATED HYDROCARBONS IN GROUND WATER SAMPLES, FORT SMALLWOOD, MARYLAND, 1974

Date (Time)	Total PCB (ppt)	Total DDT (ppt)	Chlordane Alpha (ppt)	Chlordane Gamma (ppt)	Dieldrin (ppt)
May 3 (8:45 AM)	26	6			3
Jul. 17 (10:30 AM)	1	2	1	1	1
Sept. 4 (8:30 AM)	8	1	1	1	
Oct. 16 (10:30 AM)	2	2	1	1	
Nov. 5 (12:00 noon)	9		1	1	

5.3.5 Climatological Variations

A detailed study of the climatological variations in the upper Chesapeake Bay area is beyond the scope of this investigation. However, the climatological data at Baltimore Washington International Airport are presented for interpretation and correlation purposes.

The variations of air temperature, barometric pressure, precipitation, and wind speed and direction for December 1973 through December 1974 are presented in Volume III. Table 5-8 shows the monthly averages and departures from normal of the above mentioned parameters except barometric pressure for 1974. Note that 1974 had a relatively warm winter (5°F. above normal for December) and a relatively cool summer (about 4°F. below normal). Unusually dry weather occurred in July (3.22 inches below normal precipitation), and relatively wet weather occurred in December (2.44 inches above normal). Monthly average wind speeds were all below normal for the entire year, with an extreme of 3.1 miles per hour below normal for January.

5.3.6 Variations of Water Temperature, Salinity, and Suspended Sediment

Detailed seasonal variations of water temperature, salinity, sigma-t*, and suspended sediment in the upper Chesapeake Bay are presented in Volume III. Examples are shown in Figures 5-10 and 5-11. Tables 5-9, 5-10 and 5-11 show summaries of results at three depths for three key stations—the mouth of the Susquehanna River (1A), Worton Point East (4B) and Kent Island Middle (11B).

Water temperatures at Station 1A were quite uniform throughout the water column. The maximum difference was only about 0.7°C on July 31. Seasonal changes in surface temperature ranged from 2.7°C on January 24 to 26.9°C on July 31. At Station 11B, the maximum difference was 3.8°C in temperature between surface and bottom. Its surface temperature changed from 3.0°C on January 23 to 25.2°C on July 31.

Salinity was very low and uniform at Station 1A—only 0.05 °/oo on December 14, 1973. At station 11B, surface salinity varied from 5.4 °/oo on January 23 to 12.0 °/oo on September 18. Its maximum salinity on the bottom was 15.9 °/oo on September 18.

Concentrations of suspended sediment at Station 1A reached 42.7 mg/l near the bottom on December 14, 1973. The concentrations reached as high as 80.8 mg/l near the bottom on March 20 and 92.1 mg/l on the surface on May 2 in the region of turbidity maximum near Station 4B (Schubel, 1971). At Station 11B, concentrations of suspended sediment varied from 3.0 mg/l to 5.2 mg/l on the surface and varied from 11.9 mg/l to 27.9 mg/l near the bottom.

5.3.7 Fresh Water Inputs and Tidal Variations

Figures 5-12 and 5-13 show the estimated monthly streamflow entering the Chesapeake Bay in 1974 and the annual mean flow from 1950 to 1974. The annual mean flow for 1974 was 76,900 cfs in which about 52 percent (39,900 cfs) came from the Susquehanna River. As shown in Figure 5-14, the spring freshet for 1974 occurred on April 5, when the daily mean discharge reached its maximum of 198,000 cfs (5,609 cms).

Volume III contains data for fresh water inflows from the Susquehanna River at Conowingo and from the Patapsco River at Hollofield, Maryland.

Available tide data at three key locations—Havre de Grace, Baltimore, and Matapeake are presented in Volume III. Figures 5-15 and 5-16 show the actual water level variations during the spring freshet in April and the low water period in October.

Current data at ten stations in the upper bay, which the Chesapeake Bay Institute collected for Corps of Engineers in 1971, were analyzed; results of this analysis provided additional information for the Chesapeake Bay Institute to verify their mathematical model of the Upper Chesapeake Bay. The data are recorded in Section 4 of Volume III.

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*Sigma-t is $\sigma_t = (P_t - 1)\ 1{,}000$, where P_t is the density of water at temperature t°C.

TABLE 5-8. MONTHLY AVERAGES AND DEPARTURES FROM NORMAL FOR AIR TEMPERATURE, PRECIPITATION, WIND SPEED AND DIRECTION, BALTIMORE, MARYLAND, 1974

	Air Temperature		Total Precipitation		Wind Speed and Direction			
	Averages (°F)	Departures from Normal (°F)	Precipitation (Inches)	Departures from Normal (Inches)	Resultant Vel. Dir. (mph)	Ave. Speed (mph)	Dept. From Normal	Normal Prevail. Direct.
Jan	37.9	+4.5	2.92	+0.01	2.6 WNW	7.3	-3.1	WNW
Feb	33.8	-1.0	0.94	-1.87	4.2 WNW	9.5	-1.4	NW
Mar	45.2	+2.4	4.12	+0.43	3.2 WNW	10.4	-1.2	WNW
Apr	55.3	+1.5	2.59	-0.48	5.4 W	10.2	-1.3	WNW
May	61.9	-1.8	3.58	-0.03	1.9 SW	8.9	-1.1	W
Jun	68.5	-3.9	2.84	-093	0.7 W	8.4	-0.8	WNW
Jul	76.5	-0.1	0.85	-3.22	2.7 W	8.2	-0.5	W
Aug	75.0	+0.1	5.85	+1.64	1.7 SSW	6.5	-2.4	W
Sep	67.5	-1.0	5.45	+2.33	1.7 W	6.8	-2.3	S
Oct	55.3	-2.1	1.53	-1.28	2.8 WNW	6.8	-2.8	NW
Nov	48.2	+2.1	1.39	-1.74	5.1 W	9.4	-0.5	WNW
Dec	40.3	+5.0	5.70	+2.44	2.8 WNW	8.6	-1.0	WNW

TABLE 5-9. VARIATIONS OF WATER TEMPERATURE, SALINITY, AND CONCENTRATION OF SUSPENDED SEDIMENT AT STATION 1A (BOTTOM DEPTH: 13.0 m)

Month	Dec. 73	Jan. 74	Mar. 74	May 74	Jul. 74	Sep. 74
Day	14	24	20	03	31	20
			Water Temperature (°C)			
Surface	5.09	2.73	5.28	16.08	26.93	22.68
Mid-Water	5.06	2.69	5.27	15.98	26.47	22.55
1 m Off Bottom	5.04	2.68	5.31	15.94	26.24	22.44
			Salinity (°/oo)			
Surface	0.05	0.13	0.11	0.12	0.13	0.13
Mid-Water	0.05	0.14	0.10	0.12	0.13	0.13
1 m Off Bottom	0.05	0.11	0.11	0.12	0.13	0.13
			Concentration of Suspended Sediment (mg/l)			
Surface	41.33	32.49	11.30	9.58	23.89	4.68
Mid-Water	41.57	31.27	13.32	15.03	6.90	5.09
1 m Off Bottom	42.74	33.86	14.86	16.73	29.72	12.29

TABLE 5-10. VARIATIONS OF WATER TEMPERATURE, SALINITY, AND CONCENTRATION OF SUSPENDED SEDIMENT AT STATION 4B (BOTTOM DEPTH: 12 m)

Month	Dec. 73	Jan. 74	Mar. 74	May 74	Jul. 74	Sept. 74
Day	14	23	20	2	31	19
			Water Temperature (°C)			
Surface	4.61	1.98	5.47	17.09	26.94	23.54
Mid-Water	4.89	1.65	5.26	16.90	25.59	23.14
1 m Off Bottom	5.03	1.80	5.16	16.86	25.51	23.13
			Salinity (°/oo)			
Surface	0.18	0.29	0.08	0.12	2.20	2.73
Mid-Water	0.35	1.63	0.08	0.07	3.99	3.41
1 m Off Bottom	0.47	2.52	0.09	0.08	4.54	3.48
			Concentration of Suspended Sediment (mg/l)			
Surface			41.74	92.11	12.27	13.62
Mid-Water			49.31	48.95	22.94	28.37
1 m Off Bottom			80.80	40.15	62.94	30.75

TABLE 5-11. VARIATIONS OF WATER TEMPERATURE, SALINITY, AND CONCENTRATION OF SUSPENDED SEDIMENT AT STATION 11B (BOTTOM DEPTH: 14.5 m)

Month	Dec. 73	Jan. 74	Mar. 74	May 74	Jul. 74	Sep. 74
Day	12	23	20	01	31	18
			Water Temperature (°C)			
Surface	7.65	2.96	6.05	15.14	25.23	23.10
Mid-Water	8.13	4.42	6.05	14.34	24.41	23.76
1 m Off Bottom	9.91	5.23	6.08	11.37	23.44	24.04
			Salinity (°/oo)			
Surface	10.14	5.37	6.77	5.79	10.92	11.95
Mid-Water	9.89	12.44	10.05	7.38	12.58	13.71
1 m Off Bottom	15.49	15.23	13.59	10.50	15.77	15.94
			Concentration of Suspended Sediment (mg/l)			
Surface	5.18	3.02	8.08	3.38	4.32	3.50
Mid-Water	4.20	9.28	6.91	3.72	6.18	3.61
1 m Off Bottom	27.94	14.48	14.80	11.89	18.72	19.06

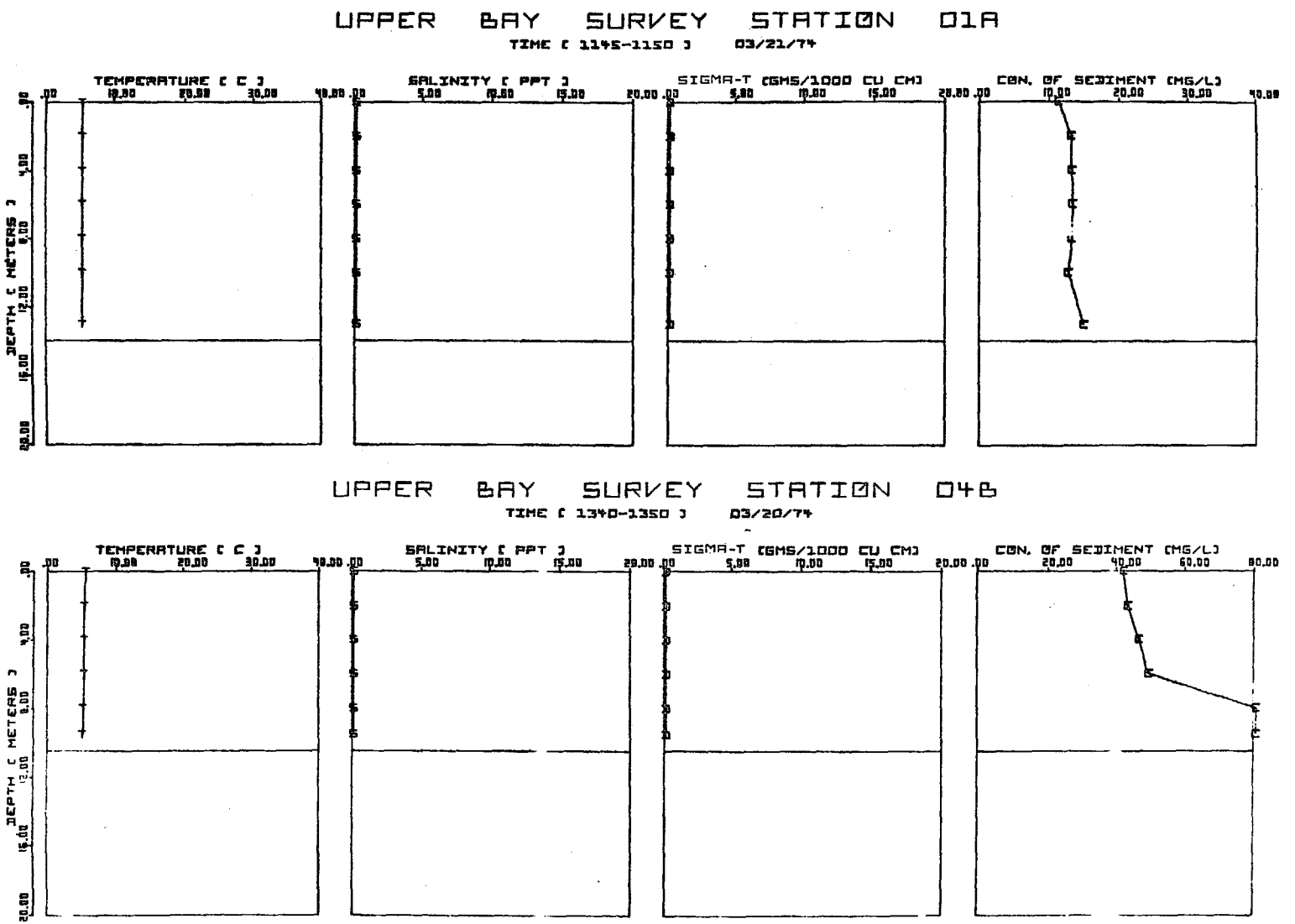

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Figure 5-10. Variations of Water Temperature, Salinity, Sigma-t and Suspended Sediment at Stations 01A and 04B

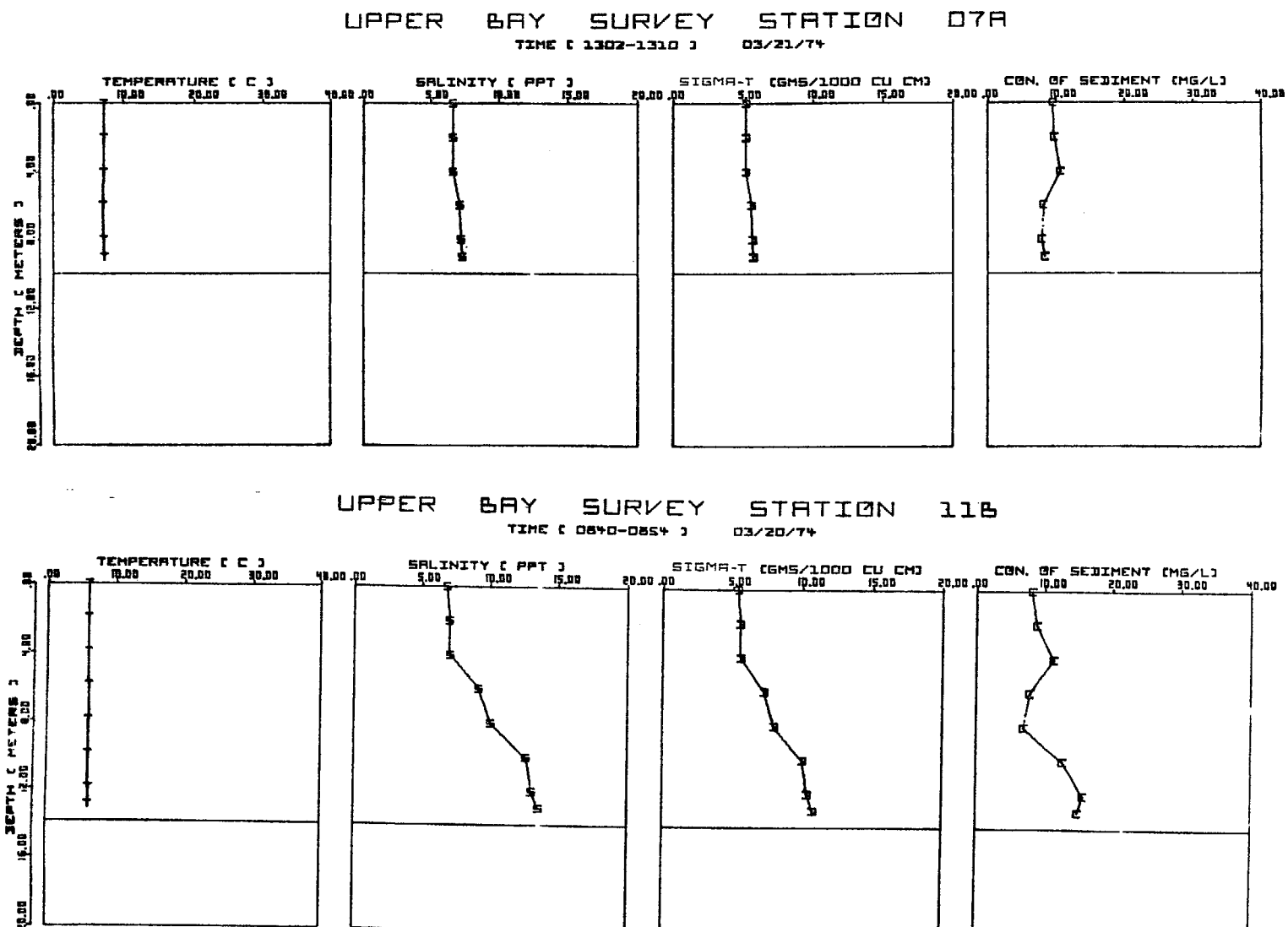

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Figure 5-11. Variations of Water Temperature, Salinity, Sigma-t and Suspended Sediment at Stations 07A and 11B

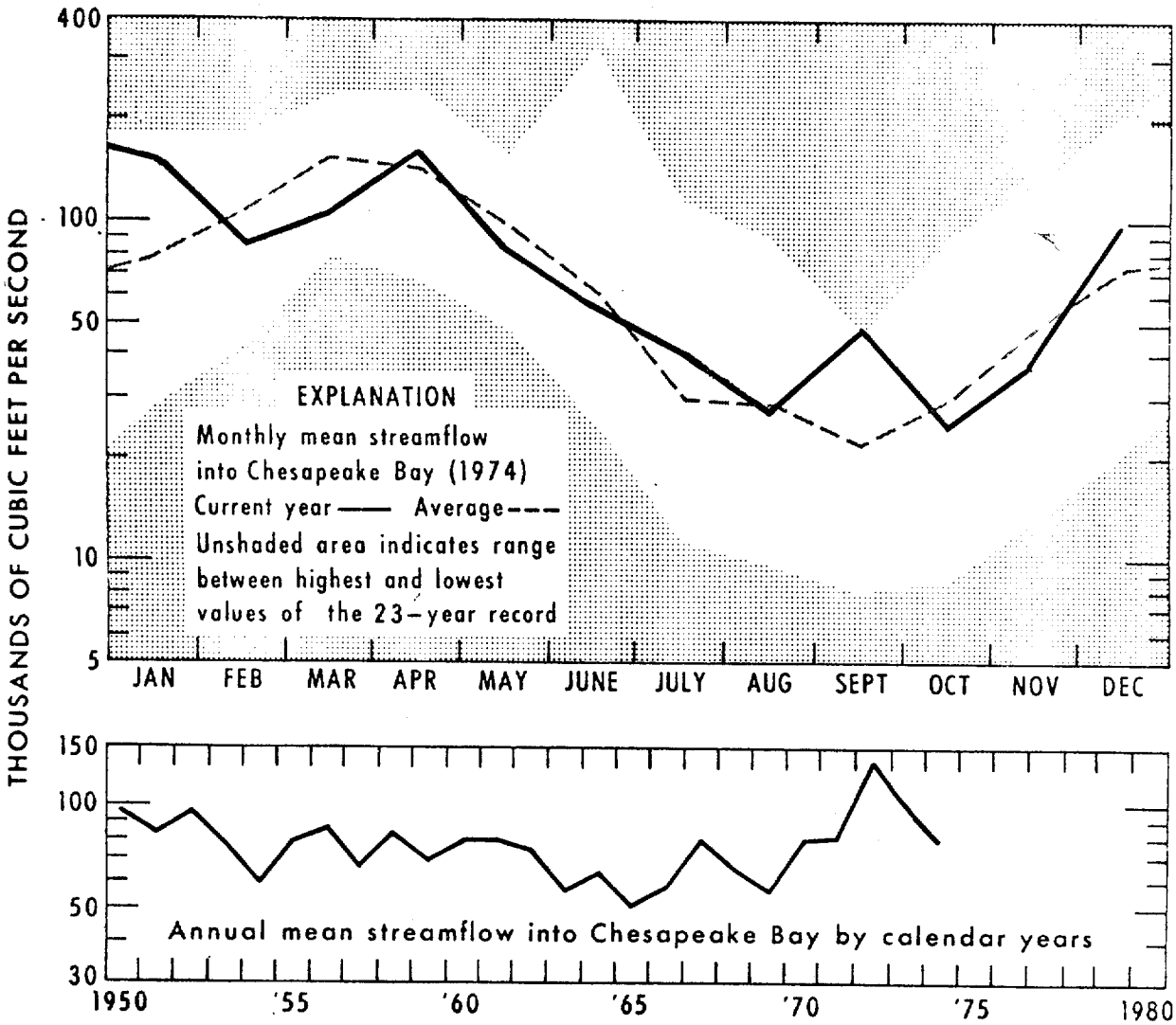

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Figure 5-12. Estimated Monthly Streamflow Entering Chesapeake Bay and Annual Mean Flow (USGS, Jan. 1975)

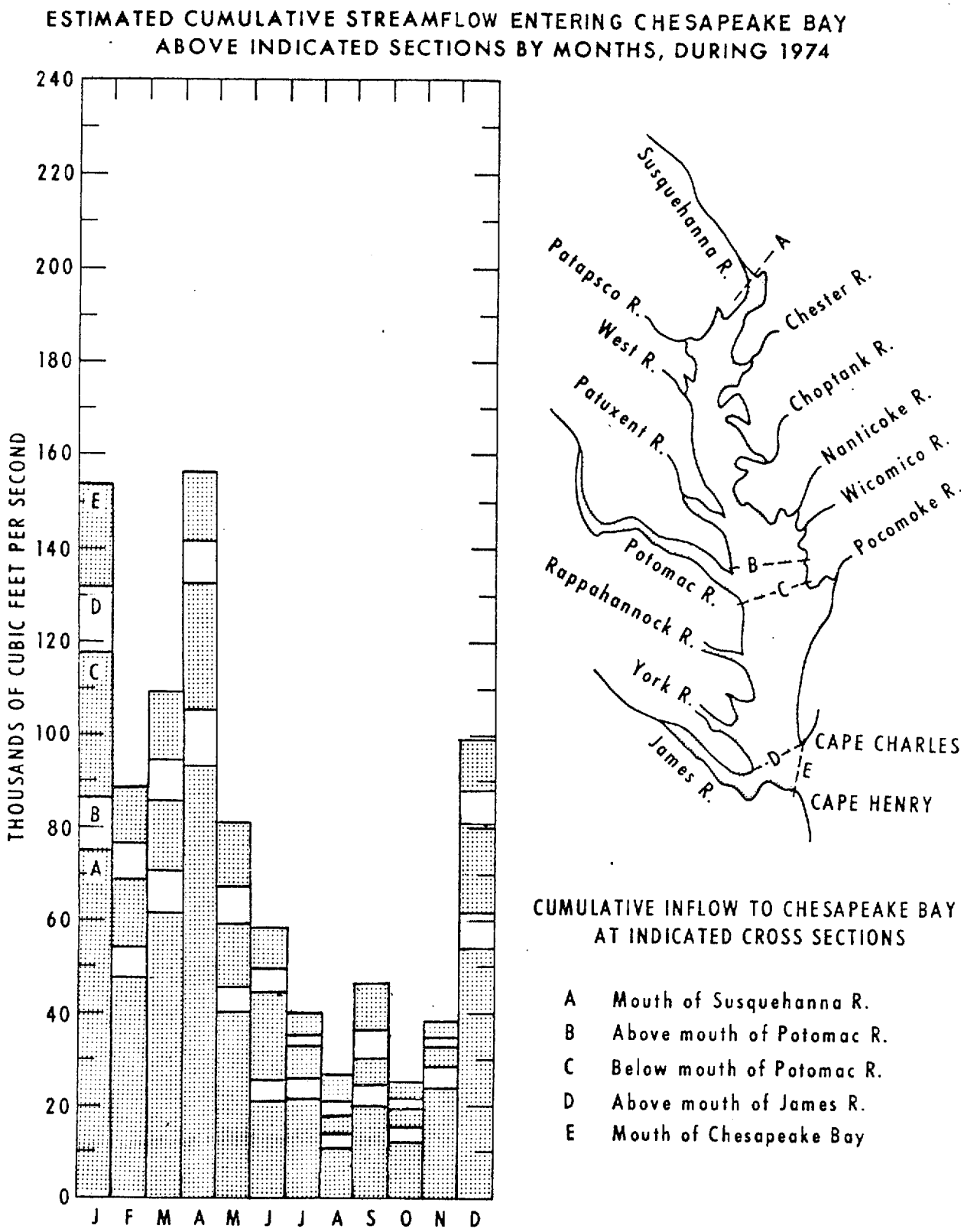

Figure 5-13. Cumulative Inflow to Chesapeake Bay, 1974 (USGS, Jan. 1975)

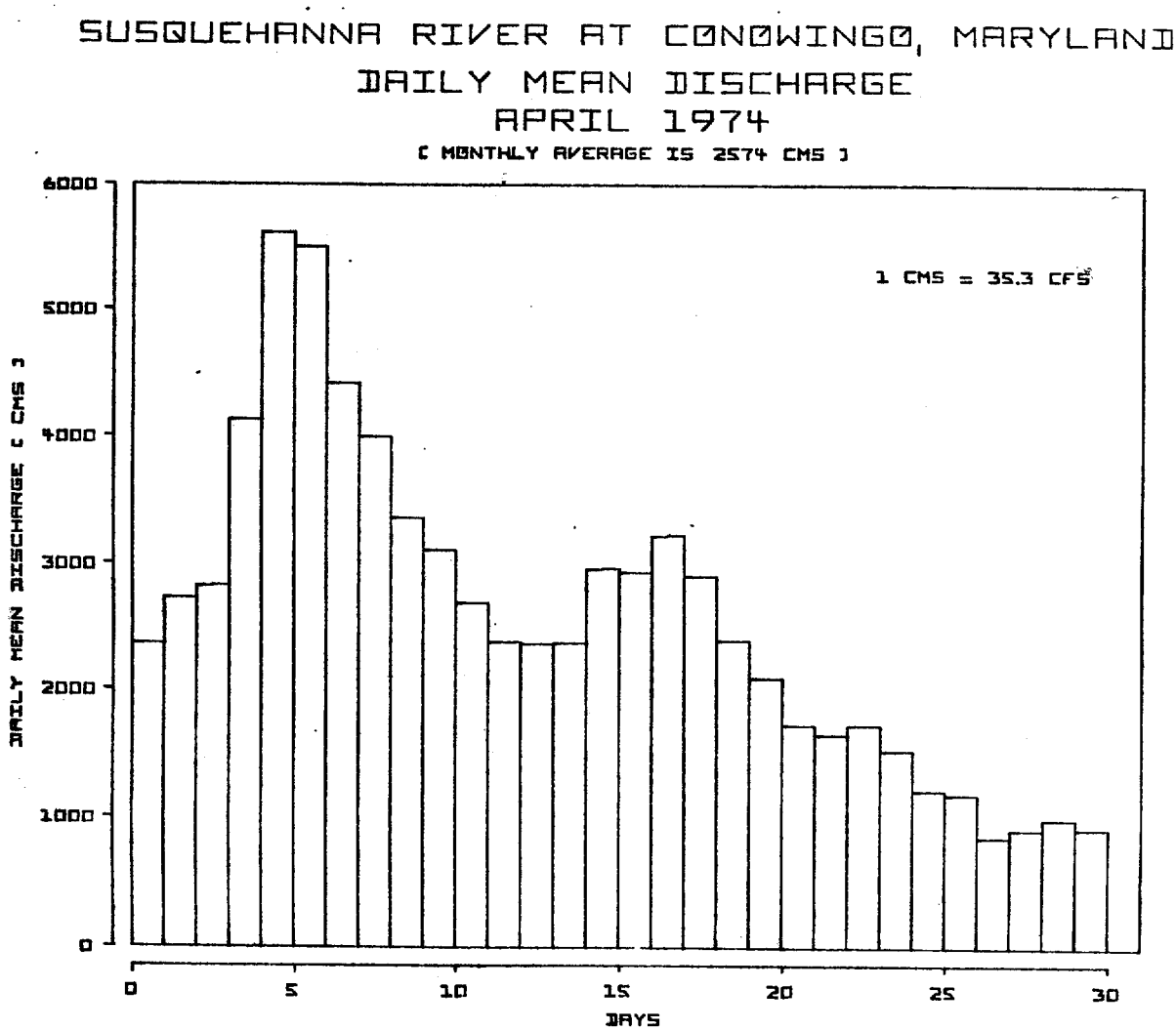

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Figure 5-14. Daily Mean Discharges for the Susquehanna River at Conowingo, Maryland

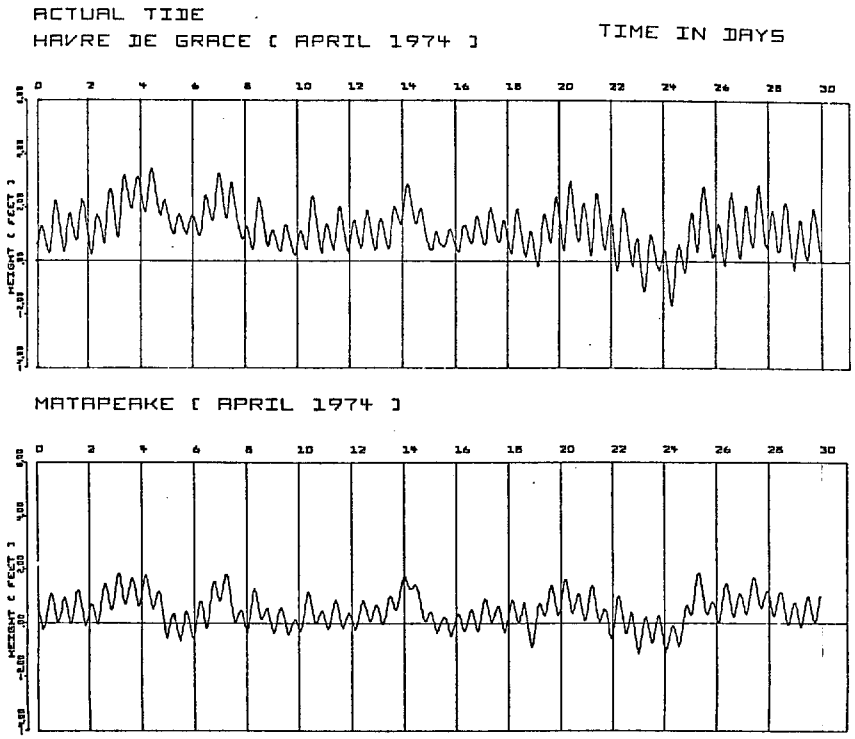

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Figure 5-15. Actual Water Level Variations at Havre de Grace and Matapeake, April, 1974

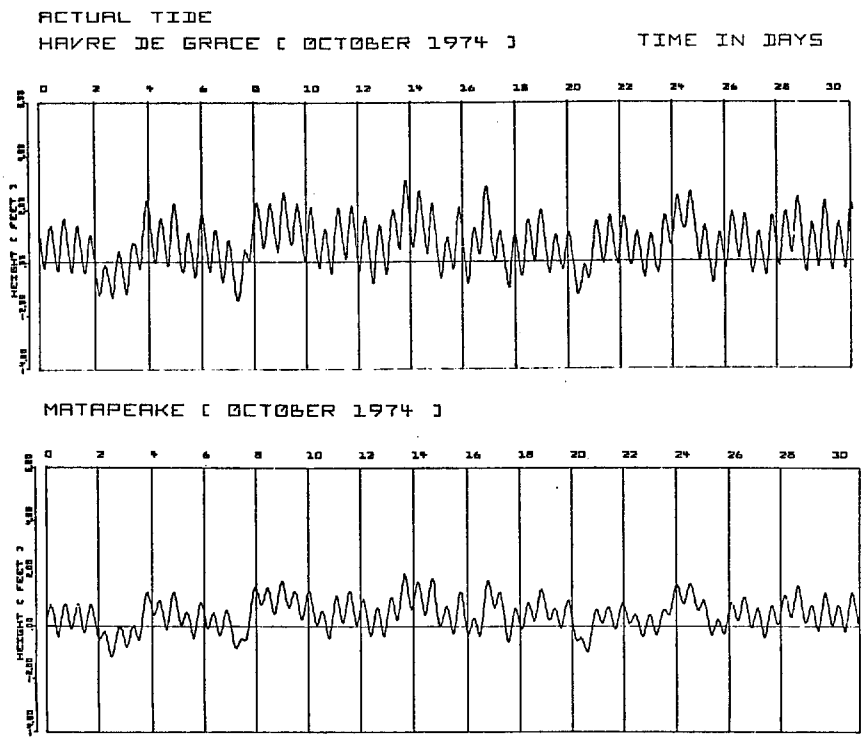

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Figure 5-16. Actual Water Level Variations at Havre de Grace and Matapeake, October, 1974

5.4 Correlation Analyses and Discussions

5.4.1 Rate of Transport of Chlorinated Hydrocarbons in the Atmosphere

Figure 5-17 shows the wind rose for July 1974 at Baltimore. The average wind velocity was 2.7 mph (1.2 meter/sec) from the west (270°).

Assume that the average concentrations of chlorinated hydrocarbons from three air samples taken in July 1974 (See Table 5-4.) represent the mean values in the atmosphere for the entire month at Sollers Point station. Then, the rates of transport for PCB and chlordane in an area of one square meter on the vertical air section for the month of July can be obtained as below:

PCB	17 $mg/m^2/month$
Chlordane	7 $mg/m^2/month$

The modes of transport of chlorinated hydrocarbons in the atmosphere are similar to the modes of other airborne pollutants. Vaporized chlorinated hydrocarbons will be partially adsorbed on particulates, transported with the prevailing winds, and deposited on land or water by particle sedimentation or rain-out. Figure 5-18 shows the summary of the resultant wind speed and direction at the Baltimore Washington International Airport for 1974. Obviously, the overall wind was from west or north-west direction. Therefore, part of the chlorinated hydrocarbons in the Chesapeake Bay could originate on the western shore areas.

5.4.2 Chlorinated Hydrocarbons Return to Earth

As an approximation, one example is worked to estimate the total return of chlorinated hydrocarbons from the atmosphere to earth through precipitation and sedimentation of particles. Basic data used for computation are as follows:

Station	Sollers Point, Maryland (Table 5-5)
Sampling Period	October 3, 7:30 AM to November 1, 8:30 AM (697 hours)
Effective Collecting Area	5 in. x 6 in. = 30 $in.^2$
Total PCB	58 ng
Total DDT	11ng
Chlordane	92 ng

For the month of October 1974 (744 hours) on a one-acre area in the neighborhood of Sollers Point, the rates of transport can be estimated as follows:

Total PCB	13 mg/acre/month
Total DDT	3 mg/acre/month
Chlordane	21 mg/acre/month

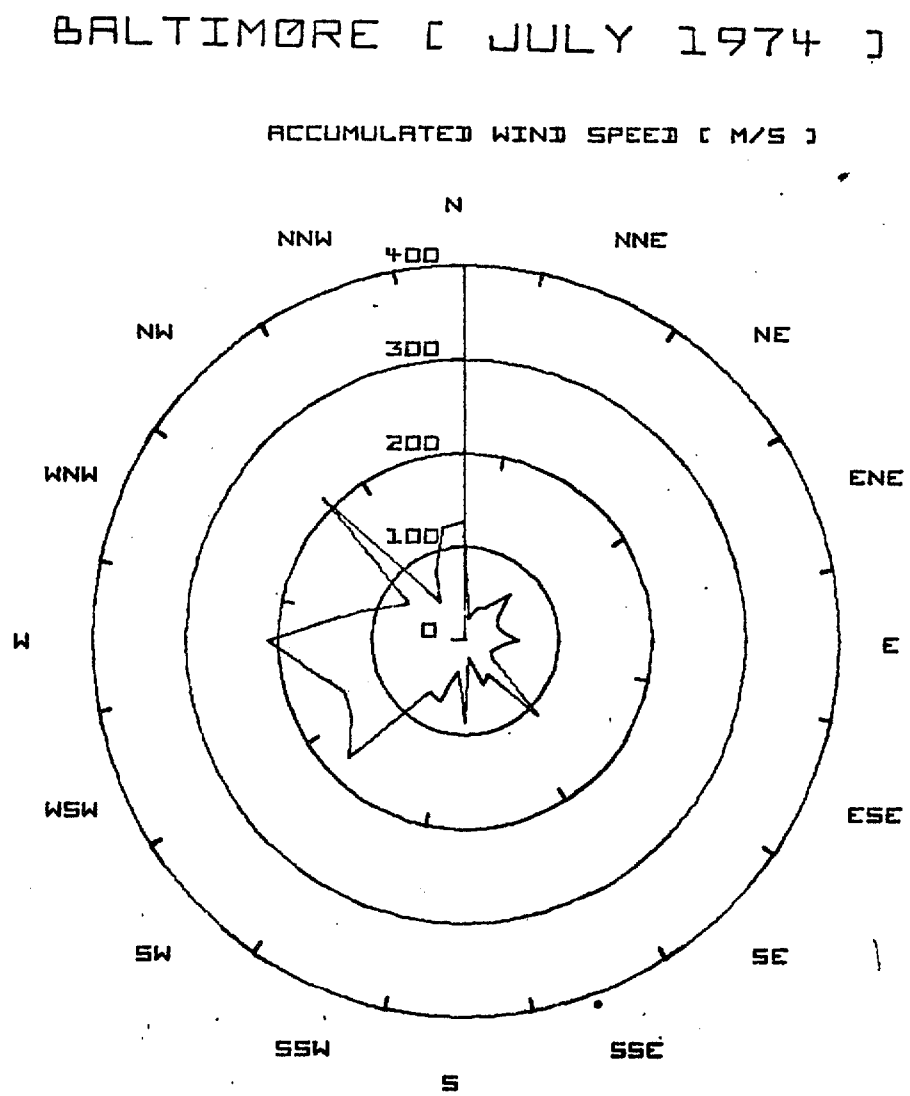

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Figure 5-17. Wind Rose for July 1974 at Baltimore Washington International Airport

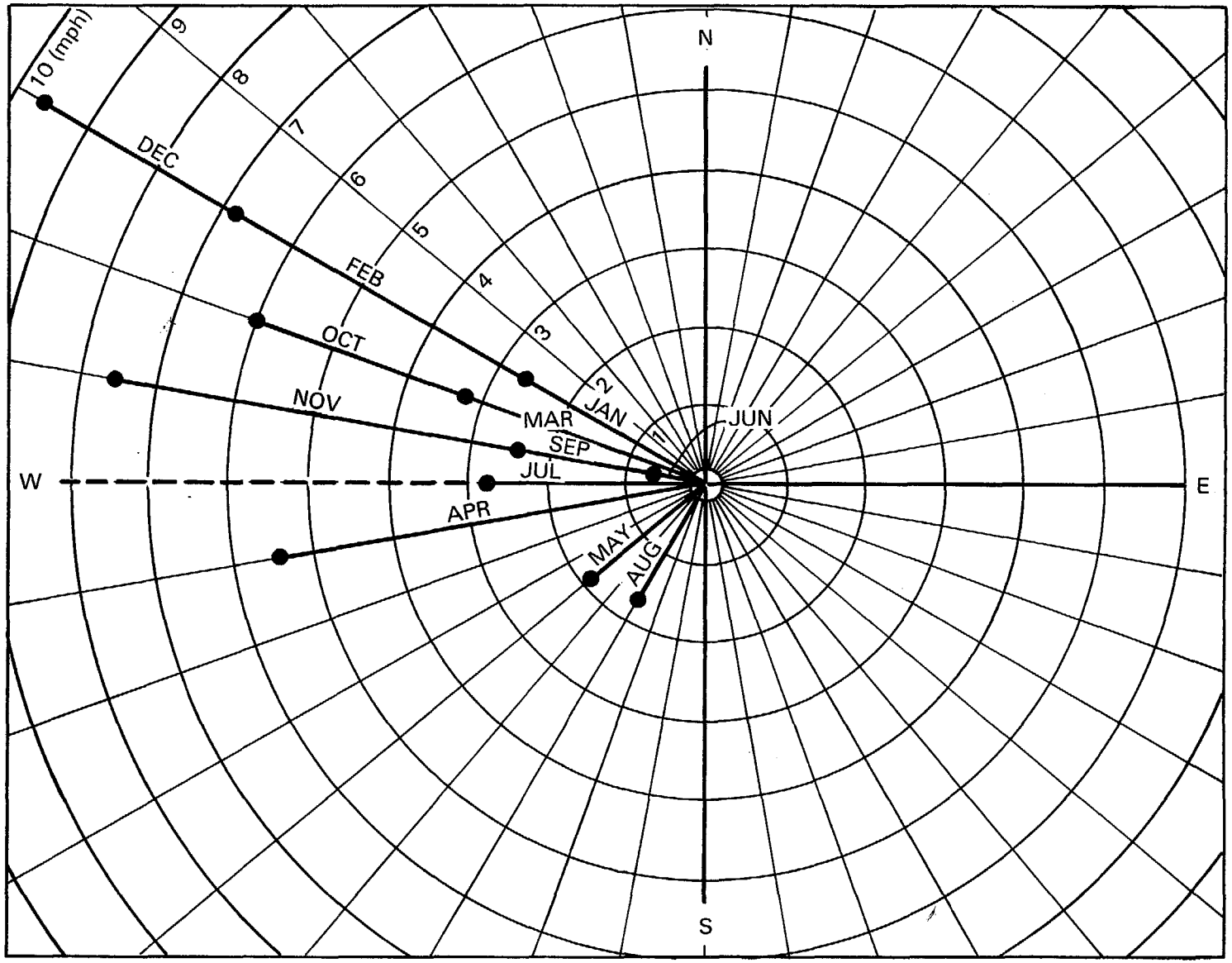

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Figure 5-18. Monthly Resultant Wind Speed and Direction at Baltimore Washington International Airport for 1974

5.4.3 Chlorinated Hydrocarbons in Storm Water Runoff and Precipitation

The Morrell Park storm water outlet was designed to carry surface runoff from the street only. During the dry weather periods, there was a small amount of ground water in the system. Figure 5-19 shows the intensity-duration curve for the storm event of March 29 and 30, 1974. The previous rain event occurred on March 21 (Figure 5-20) and was followed by a seven-day dry period. As shown in Figure 5-19, two samples were collected, one at 8:30 AM (the rain started at 12:00 noon) and one at 6:30 PM on March 29. Significant changes in chlorinated hydrocarbon concentrations were revealed in Figure 5-19. Total PCB increased from 12 ppt to 580 ppt; total DDT decreased from 12 ppt to 5 ppt, and chlordane increased from 11 ppt to 63 ppt. Results obviously suggest that the street runoff contained high concentrations of PCB's and chlordane washed from the area.

5.4.4 Spring Freshet: Inputs of Sediments and Chlorinated Hydrocarbons

The Susquehanna River, the upper Chesapeake Bay's only major source of fresh water, discharges more than 97 percent of the total fresh water and fluvial sediment into the upper portion of the Bay (Schubel, 1969). During the first week of April 1974, the average discharge was 139,200 cfs (3,-943 cms), See Figure 5-14. If it is assumed that the average concentrations of suspended sediment and chlorinated hydrocarbons at Station 2B (see Figure 5-3) for April 3 and 4 were the same as the values for April 1 through 7 at Conowingo gauging station, then the inputs of sediment and chlorinated hydrocarbons can be estimated as:

Suspended Sediment	35.9 mg/l
Total PCB	10.1 ppt
Total DDT	1.0 ppt
Chlordane	0.3 ppt

For the first week of April 1974, the total volume of discharge was 2.39×10^9 m^3. Thus, the approximate rates of input are:

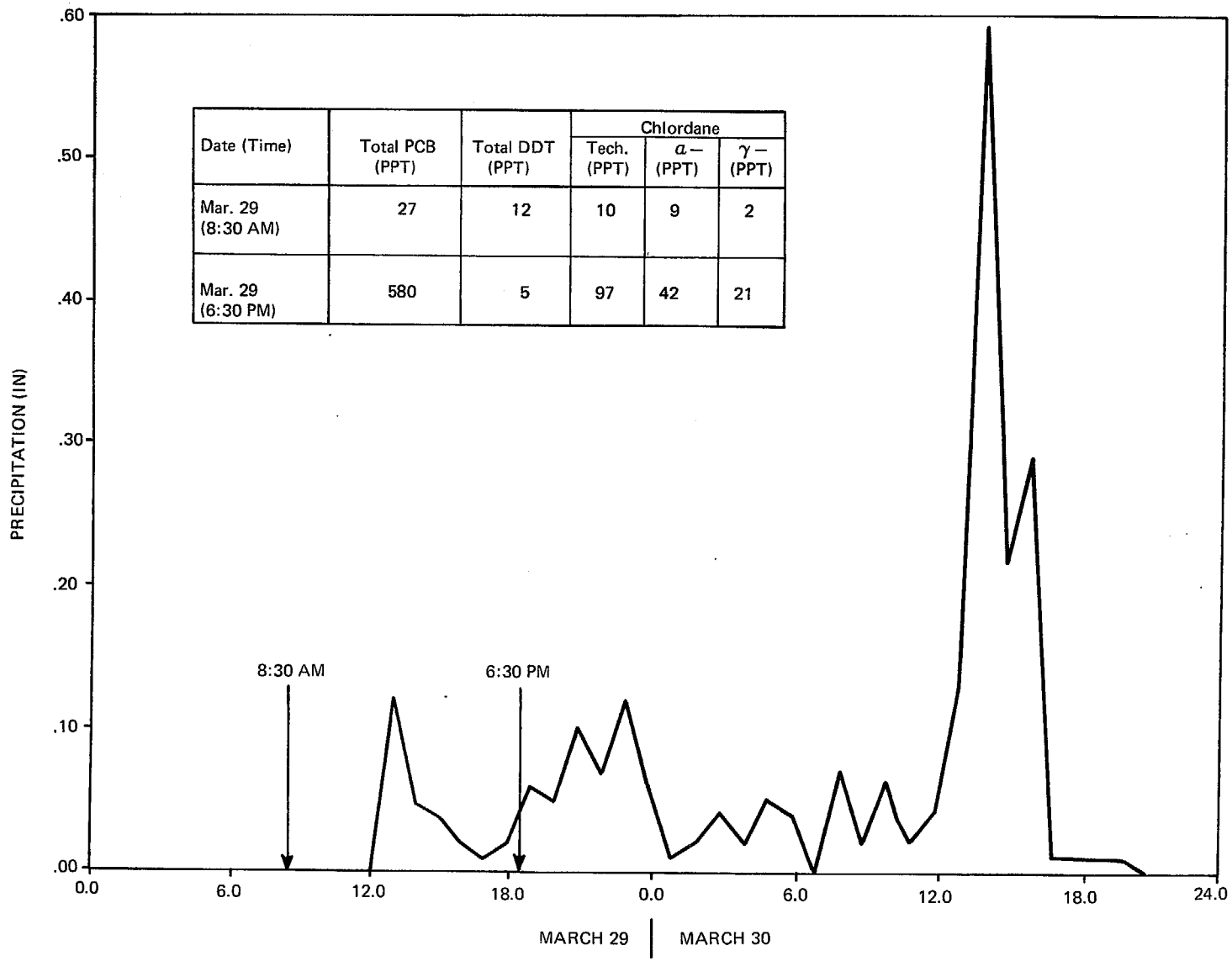
Suspended Sediment	85,703 metric tons/week
Total PCB	24 kg/week
Total DDT	3 kg/week
Chlordane	0.8 kg/week

Based on the available data-averaged concentrations of chlorinated hydrocarbons in the suspended sediments at Station 1A were:

Total PCB	0.633 ppm
Total DDT	0.035 ppm
Chlordane	0.030 ppm

Figure 5-21 shows the monthly inputs of suspended sediments from the Susquehanna River into the Chesapeake Bay. If it is assumed that these sediments carried the same concentrations of chlorinated hydrocarbons as the sediments at Station 1A, the total inputs of chlorinated hydrocarbons to the bay can be estimated as:

Total Suspended Sediment	800,000 metric tons for 1974
Total PCB	506 kg/year
Total DDT	28 kg/year
Chlordane	24 kg/year



Date (Time)	Total PCB (PPT)	Total DDT (PPT)	Chlordane		
			Tech. (PPT)	$\alpha-$ (PPT)	$\gamma-$ (PPT)
Mar. 29 (8:30 AM)	27	12	10	9	2
Mar. 29 (6:30 PM)	580	5	97	42	21

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Figure 5-19. Precipitation and Concentrations of Chlorinated Hydrocarbons in Storm Water

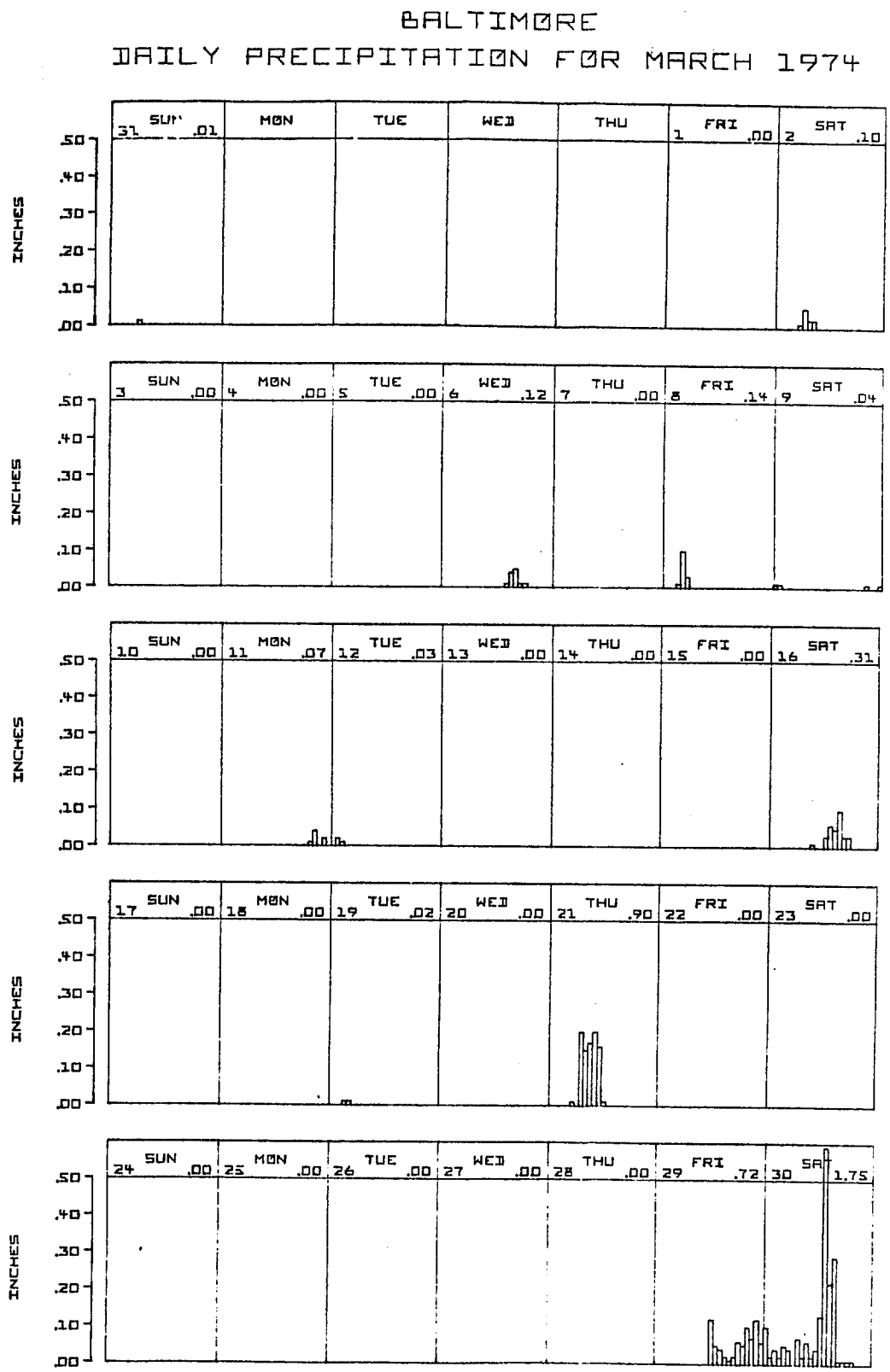

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Figure 5-20. Hourly Precipitation at Baltimore, March, 1974

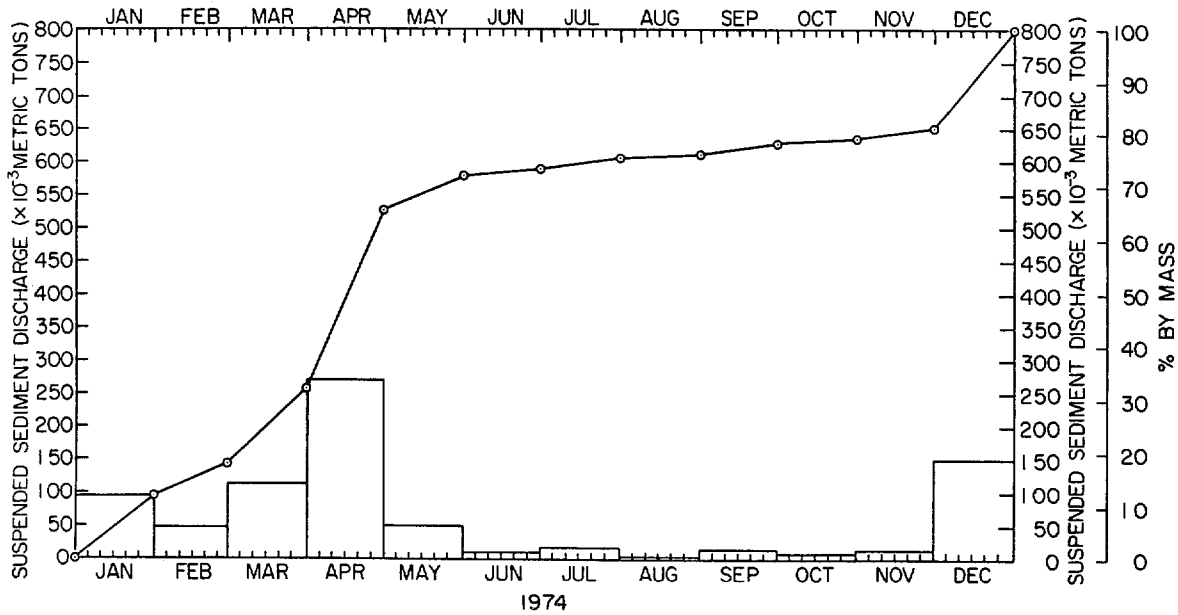

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Figure 5-21. Estimated Monthly Inputs of Suspended Sediment from Susquehanna River into Chesapeake Bay, (Schubel, 1975)

5.5 Conclusions

The hydrological and meteorological investigations of this study were focused primarily on the understanding of distributions and transport mechanisms of chlorinated hydrocarbons in the upper Chesapeake Bay environment. It is no surprise to note that the major phases of the hydrologic cycle—evaporation, precipitation, surface runoff, ground water flow, and the receiving water body are all contaminated more or less with chlorinated hydrocarbon residues. Important conclusions from this study can be summarized as follows:

- Chlorinated hydrocarbons in the atmosphere are mainly in the vapor state. Only 9 percent of the PCB and 18 percent of the chlordane were found in the suspended particulate matter trapped by the Gelman filter. The highest levels of chlorinated hydrocarbons in the atmosphere were 9 ng/m^3 PCB, 3 ng/m^3 chlordane, and less than 1 ng/m^3 DDT. For the month of July 1974, estimated rates of transport of PCB and chlordane over the Sollers Point neighborhood were:

PCB	17 mg/m^3/month
Chlordane	7 mg/m^2/month

- Airborne chlorinated hydrocarbons return to earth through precipitation and aerial fallout. Toxaphene as high as 283 ppt was detected in Fort Smallwood rain samples. At Sollers Point, 130 ppt PCB and 180 ppt chlordane were found in the rain samples. For the month of October 1974, estimated transports of chlorinated hydrocarbons from air to earth in the Sollers Point area were:

PCB	13 mg/acre/month
DDT	3 mg/acre/month
Chlordane	21 mg/acre/month

- Significant changes in chlorinated hydrocarbon concentrations were found in a storm water system before and after a rain storm. At Morrell Park station, it was found that PCB increased from 12 ppt to 580 ppt, DDT decreased from 12 ppt to 5 ppt, and chlordane increased from 11 ppt to 63 ppt for the rain storm of March 29, 1974.
- Concentrations of chlorinated hydrocarbons in ground water samples were less than those in rain water and storm water samples. Maximum levels at Fort Smallwood were 26 ppt PCB, 6 ppt DDT, and 2 ppt chlordane.
- In 1974, the spring freshet from the Susquehanna River occurred in the first week of April. Its daily mean discharge reached 5,609 cubic meters per second at Conowingo on April 5. Estimated inputs of sediment and chlorinated hydrocarbons at the month of the Susquehanna River for this period were:

Suspended sediments	85,703 metric tons/week
PCB	24 kg/week
DDT	3 kg/week
Chlordane	0.8 kg/week

Total inputs of suspended sediment and chlorinated hydrocarbons for the entire year were estimated as:

Suspended sediments	800,000 metric tons/year
PCB	506 kg/year
DDT	28 kg/year
Chlordane	24 kg/year

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CHAPTER 6

BIOCHEMISTRY

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ABSTRACT

The Upper Bay Survey was a multidisciplinary program to study the rates, routes, sources, sinks, and reservoirs of chlorinated hydrocarbons (CHC's) in the upper Chesapeake Bay. Samples of zooplankton, suspended sediments, bottom sediments, benthic organisms, water, rain, air, ground water, and storm sewer effluent were analyzed for chlorinated hydrocarbons, including chlorinated pesticides and polychlorinated biphenyls (PCB's). Chlordane (a pesticide banned in 1975), PCB's (a group of industrial chemicals) and DDTR (the total residues of the pesticide DDT, which was banned in 1972) were found in all types of samples analyzed. Traces of toxaphene (a chlorinated pesticide) were found in some samples taken in the area of Baltimore harbor.

The sediments in Baltimore harbor were found to contain the highest levels of CHC's in upper bay sediments, although, the concentrations of CHC's in the bottom sediments in the bay were two to three times higher, on the average, than those found previously in the Chester River. The suspended sediments in Baltimore harbor contained the highest levels of PCB, chlordane, and DDTR (3.8, 0.34, and 0.30 ppm, respectively). The highest PCB and DDTR values in zooplankton were found at the head of the bay near the mouth of the Susquehanna River (PCB, 7.5 ppm; DDTR, 4.2 ppm). The chlordane values in zooplankton usually were highest in Baltimore harbor, the highest value being 0.14 ppm.

Positive correlations were found between the concentration of suspended sediments in the water and the concentration of CHC's in the water column on suspended sediments and between the zooplankton biomass in the water and the CHC concentration in the water column associated with zooplankton. Apparently, the CHC's are transported while associated with suspended sediments. When zooplankton blooms occur, CHC's pass from the suspended sediment to the zooplankton with the zooplankton bio-concentrating the CHC's five to eight times over the levels found in the suspended sediments.

The Susquehanna River appears to be the major source of CHC's to the upper Chesapeake Bay. Although, Baltimore harbor appears to have localized sources of CHC's which cause high levels of PCB, chlordane, and DDTR in this harbor, it is not clear whether the harbor contributes important quantities of PCB and DDTR to the bay. The data do suggest that the net transport of substantial quantities of chlordane from the harbor to the bay does occur.

TABLE OF CONTENTS

Section		Page
6.0	BIOCHEMISTRY	6-1
6.1	Introduction	6-1
6.2	Analytical Methods	6-1
6.2.1	Sample Collection	6-1
6.2.2	Gas Chromatographic Methods	6-4
6.3	Results	6-5
6.3.1	Bottom Sediments	6-5
6.3.2	Suspended Sediments	6-9
6.3.3	Zooplankton	6-9
6.3.4	Benthic Organisms	6-16
6.3.5	Laboratory and *In Situ* PCB Exposure Experiments	6-16
6.3.6	Other Samples	6-16
6.4	Discussion	6-18
6.5	Conclusions	6-29
6.6	References	6-30

LIST OF FIGURES

Figure		Page
6-1	Bottom Sediment Sampling Locations	6-6
6-2	PCB in Bottom Sediments	6-7
6-3	DDTR in Bottom Sediments	6-8
6-4	Average PCB Concentrations by Stations	6-10
6-5	Average Chlordane Concentrations by Station	6-11
6-6	Average DDTR Concentrations by Station	6-12
6-7	Average PCB Concentrations by Date	6-13
6-8	Average Chlordane Concentrations by Date	6-14
6-9	Average DDTR Concentrations by Date	6-15
6-10	Concentration of Chlorinated Hydrocarbons in Water on Suspended Sediments	6-22
6-11	Chlorinated Hydrocarbons in Water on zooplankton	6-24
6-12	Zooplankton Biomass vs Chlorophyll-a Concentration	6-25

LIST OF TABLES

Table		Page
6-1	Chlorinated Hydrocarbons in Benthic Organisms	6-17
6-2	Chlorinated Hydrocarbons in Suspended Sediments	6-19
6-3	Average Chlorinated Hydrocarbons Found in the Upper Chesapeake Bay	6-19
6-4	Types and Levels of Chlorinated Hydrocarbons Found in the Chester River	6-20

6. BIOCHEMISTRY

6.1 Introduction

The Chester River Study set the stage for the Upper Bay Survey. During the Chester River Study, it was learned that certain chlorinated hydrocarbons (CHC's) were present in the lower Chester River, and that these CHC's probably entered the Chester River from the upper Chesapeake Bay via the suspended sediments. The Upper Bay Survey then was implemented with the overall objective of studying the rates, routes, sources, sinks and reservoirs of chlorinated hydrocarbons in the upper Chesapeake Bay. A basic assumption used in the design of this program was that in the relatively turbid waters of the bay, the water insoluble CHC's would be adsorbed to suspended sediments in the water column rather than being present in solution. In addition, it was felt that CHC's probably could pass from the non-biological medium (adsorbed on suspended particulates) to the biological medium in the water column (the phytoplankton-zooplankton food chain).

Although various types of samples were collected and analyzed for CHC's, the bulk of the field samples fell into four categories which will be presented and discussed in this section—bottom sediments, suspended particulates, zooplankton, and benthic organisms. Data from the remaining samples will be presented and discussed in the appropriate sections.

6.2 Analytical Methods

6.2.1 Sample Collection

6.2.1.1 Sediment Samples

Each bottom grab sample was spread out in a hexane-rinsed nine-inch disposable aluminum pie plate and allowed to air-dry completely (usually in one to two weeks). The dried sediments then were ground (using either a mortar and pestle or a Lamair Instruments Model 150 jaw crusher) and placed in a hexane-rinsed Mason jar fitted with an aluminum foil-lined cap.

Each ground sample was sieved through a one-millimeter screen (No. 18 sieve), and 50 grams were placed in a cleaned and checked* fritted glass thimble in a 500-milliliter Soxhlet extractor. A wad of pre-extracted and baked glass wool had been placed in the thimble to prevent the sediment from clogging the frit. The sample was then extracted for 12 hours with a 2:1 hexane-acetone solution, and the extract was concentrated to about ten milliliters in a checked 500-milliliter Kuderna-Danish concentrator. The sample was then ready for clean-up.

Chromatographic grade (80-325 mesh) alumina which had been heated to 300°C overnight, cooled, and de-activated with five percent (weight to weight) distilled water was prepared fresh every two to three days. Thirty grams was placed on a pre-extracted glass wool plug in a Chromaflex chromatography column and topped with about five grams of anydrous sodium sulfate. The column then was washed with about 250 milliliters of hexane. After running the sample extract into the column, the chlorinated hydrocarbons were washed through with 225 milliliters of six percent (volume to volume) diethyl ether in hexane into the previously used Kuderna-Danish Concentrator (a clean bottom tube was attached) at a flow rate of five milliliters per minute. This eluate was then concentrated to about five milliliters and placed in a clean disposable culture tube with a teflon-lined screw cap.

About two milliliters of 1:1, 20-percent fuming sulfuric acid-concentrated sulfuric acid mixture was added to the alumina cleaned extract. The tube then was shaken for about a minute and allowed to settle for approximately three hours.

The supernatant was drawn off with a Pasteur pipet and placed in a 12-milliliter Kontes micro-concentrator sample tube. The acid layer was re-extracted with two four-milliliter portions of hexane. All of the extracts were combined in the micro-concentrator tube and then concentrated (using a Kontes tube heater, Part K-720000) to about five milliliters and placed in a culture tube.

The bottom sediment samples were contaminated with sulfur which causes interference with the chromatographic analysis. Therefore, samples were treated two or three times with elemental mercury to remove the sulfur (Goerlitz and Law, 1971). Finally, the samples were put in tared culture tubes and weighed, and the volumes were calculated using 1.5 as the density of hexane.

The suspended sediment filters wers air dried for three to five days. Then they were torn into small pieces and extracted for eight hours in a Soxhlet extractor with 300 milliliters of 2:1 hexane acetone, a wad of pre-extracted glass wool having been placed in the extractor. The extract then was concentrated in a 500-milliliter Kuderna-Danish concentrator to about five milliliters and placed in a culture tube. This extract then underwent the tube acid treatment described above. The final volume was taken to one milliliter or less, and again the volume was calculated by weight using the density of hexane.

6.2.1.2 **Biological Samples**

Oysters and Soft Shell Clams-The shellfish were shucked, drained, and homogenized to a puree in a Vortis homogenizer. Approximately ten grams was placed in a hexane-rinsed aluminum weighing pan and weighed to three significant figures. The sample then was ground to a dry powder with anhydrous sodium sulfate with a mortar and pestle. The samples were Soxhlet extracted as described in the bottom sediment procedure. If, after being concentrated to less than ten milliliters, the extract appeared to contain little lipid and/or insoluble material, the extract underwent the small-tube acid clean-up described above. Otherwise, the extract received a fuming sulfuric acid treatment as described in the next paragraph (Munson, 1972).

A mixture of nine milliliters of 20 to 30 percent fuming sulfuric acid and nine milliliters of concentrated sulfuric acid was added slowly while stirring to 30 grams of Celite (Johns-Manville 545AW). About 200 milliliters of petroleum ether was immediately stirred into the acid-wetted Celite, and the resulting slurry was added to a 90 millimeter sintered-glass filter funnel and packed down by pressing the upper surface with a small glass beaker. After draining off the excess petroleum ether above the surface of the packed column with vacuum, the column was washed with a second 200 milliliters of petroleum ether which was discarded after removal from the column. Care was taken not to run the column dry.

The concentrated lipid extract then was added to the top of sulfuric acid-Celite column and slowly drawn down through it with a gentle vacuum. The acid-resistant chlorinated hydrocarbons were subsequently washed off the column with 400 milliliters of petroleum ether and the

*See Section 6.2.1.5 for cleaning and checking procedures.

filtrate concentrated to about five milliliters in a Kuderna-Danish evaporative concentrator. The resulting sample volume was measured, and the sample was stored in a 10-milliliter culture tube sealed with a teflon-lined screw cap.

Considerable charring and discoloration usually occurred on the upper surface of the sulfuric acid-Celite column, but the concentrated filtrate was nearly always completely clear and colorless. However, if the lipid extract had not been concentrated enough to boil off all the acetone prior to addition to the acid column, the char and discoloration would pass through the column and contaminate the filtrate sufficiently to require that the acid clean-up procedure be repeated.

Crabs-The legs and carapace were removed and discarded from each frozen crab. The total internal contents were scraped into a blender and reduced to a homogeneous puree. In the case of small crabs, several were mixed together. About ten grams of the puree were weighed, extracted, cleaned up, and analyzed exactly as described for shellfish.

Plankton-The frozen samples were defrosted as needed. Those samples which weighed ten grams or less (Wet and dry weights were determined as part of the biology program.) were placed directly into a mortar and ground to a powder with sodium sulfate. A ten-gram fraction was taken from larger samples and ground. The powder was then extracted for eight hours in a Soxhlet extractor with 300 milliliters of 2:1 hexane acetone solution. A wad of pre-extracted glass wool was placed in the Soxhlet to prevent the sample itself from siphoning over. The extracts were concentrated and acid-treated as described for the bottom grab sediments.

Extremely small plankton samples (0.1 grams or less) were shaken with five milliliters of hexane in a culture tube and tube acid treated directly.

6.2.1.3 **Water Samples**

Water samples such as rain and ground water were collected in five-gallon glass bottles. If the water level was well below the neck of the bottle, hexane-extracted distilled water was added to raise the level. The bottles were placed on magnetic stirrers, and hexane was added to completely fill the bottle (about 50 milliliters). The water was extracted by drawing hexane down through it for about one hour. The hexane then was pipetted off; the procedure was repeated four more times, and the extracts were collected. The extracts were passed through sodium sulfate before concentration in a 500-milliliter Kuderna-Danish concentrator to about five milliliters. These extracts then were submitted to the small tube acid treatment.

Samples which contained a lot of sediment were filtered through a pre-extracted Gelman 142-millimeter glass-fiber filter before extraction.

6.2.1.4 **In Situ Experiment Samples**

The preparation and analysis for the *in situ* experiment was much simpler than that for the majority of the program. Three types of samples–suspended sediments, clams and oysters, and water—were analyzed only for PCB 1254.

The wet suspended sediment filters were placed in 250-milliliter Erlenmeyer flasks and extracted by boiling with three 150-milliliter portions of 2:1 hexane acetone solution. The extracts were concentrated, placed in culture tubes, and small tube acid treated as described above.

The clams and oysters were prepared for extraction exactly as described above, except each entire animal was ground to a dry powder with anhydrous sodium sulfate rather than an aliquot of the homogenate. The dry powder sample was placed in a 19 x 300-millimeter integral reservoir chromatography column with a pad of glass wool at the bottom. The sample was extracted by passing 225 milliliters of hexane-acetone (2:1 solution) through the column at a rate of about five milliliters per minute (Hesselberg and Johnson, 1972). The extract was collected in a 250-milliliter flask and concentrated to 40 milliliters as described above. The concentrated extract was cleaned-up, using the acid-Celite column as described above.

The water samples also were prepared as described, except that only three hexane extractions were made.

The Hewlett-Packard 5700A chromatograph with the computing integrator was used for all analyses, and a 4% SE-30/6%-SP2401 column packing was employed. There were relatively few conflicting peaks as the PCB 1254 concentration was so high. This fact, coupled with the use of the integrator, made quantitation simple and rapid.

6.2.1.5 **Glassware**

Glassware contamination posed some problems. Solvent blanks run in glassware which was simply washed with Alconox and water gave a great many interfering peaks when checked by gas chromatography. Therefore, a more extensive cleaning procedure was employed routinely. Glassware such as Kuderna-Danish concentrators, round bottom flasks used with the Soxhlet extractors, and new culture tubes were soaked in chromic acid for several hours and then rinsed with tap water. The other glassware was washed with soap and water. All pieces were rinsed consecutively with hexane, petroleum ether, and acetone before baking overnight at 250°C.

Blanks were run on concentrators and Soxhlet extractors (with glass thimbles) before use. Soxhlets often had to be run empty for several nights before they became clean, especially after samples of bottom sediments had been extracted.

It was found that previously clean glassware, if allowed to remain idle in the laboratory for several weeks, became contaminated, requiring that the cleaning process be repeated. Cleaned culture tubes were stored in aluminum foil-covered beakers. Flasks, graduated cylinders, and other glassware were well rinsed with solvents before use.

6.2.2 **Gas Chromatographic Methods**

In light of the relatively low levels of CHC's in most of the samples, qualitation and quantitation was difficult even though great care was taken to minimize contamination of samples during the preparation and clean-up. A multitude of materials can cause electron-capture detector deflections and artifact peaks due to unknown compounds. Compounds other than known chlorinated hydrocarbon environmental contaminents, indeed, were present on many of the chromatograms. In many samples, therefore, the key task in qualitation and quantitation of the chlorinated hydrocarbons of interest was to distinguish the artifact peaks and avoid confusing them with the compounds to be quantitated.

6.2.2.1 **Instrumental Parameters**

All analyses were done using either a Nuclear Chicago 5000 series dual-column, gas-liquid chromatograph equipped with two nickel-63 electron-capture detectors or a Hewlet Packard 5700A series gas-liquid chromatograph with a pulsed, nickel-63 electron-capture detector. The Hewlett Packard chromatograph also was equipped for the last half of the program with a Spectra-Physics Computing Integrator for Chromatography, System 1.

The fact that the two four-millimeter (inside diameter) columns in the Nuclear Chicago 5000 were not in dual ovens posed some difficulties in the column packing selection. Primarily, three packings were used in this instrument, all at 200°C. They were 4% SE-30/6%-SP-2401 on 100/120 Supelcon AW-DMCS (Supelco No. 01-1948) or 1.5%-SP2250/1-95%-SP2401 on 100/120 Supelcon AW-DMCS used in 183-centimeter long, glass, U-shaped columns, and 5% DC 200/2.5%QF1 on 80/100 mesh Chromosorb W-HP (Chemical Research Services PA-3) used in a 122-centimeter U-shaped column. Although, nitrogen gas flow was adjusted to give each column a suitable retention time for aldrin, the flow was maintained between 50 and 100 ml/min to ensure proper detection performance. The injection ports were at 225°C, and the detectors were at 280°C. Detector voltages were adjusted so that 0.16 nanograms of aldrin gave a response of 60 percent of full scale at an attenuation of 4×10^{-10} amps. Where necessary, samples were diluted to give responses that fell within the linear response ranges of the detectors as determined by running standardization curves with varying concentrations of standards.

The Hewlett Packard chromatograph was equipped with only one detector. Either a 4% SE 30/6%-SPS401 on a 80/100 mesh Supelcon support run at 205°C or a 5%-DC-200/2.5% QF1 on 80/100 mesh Chromosorb W-HP at 215°C was used. The columns were 183 centimeter coiled glass; the injection port heat was 250°C, and the detector heat was 300°C. The 95% argon/5% methane gas flow was set so that aldrin gave a retention time of about five minutes. This instrument did not necessitate diluting the samples, because the detector response was shown to be linear over nearly the entire range of the instrument.

6.2.2.2 Qualitation and Quantitation

The peaks on the chromatographic traces were identified by comparing the retention times relative to aldrin. The peaks were matched to the major peaks of the standard materials. Each polychlorinated biphenyl (PCB) had a least one major peak which made it distinctive from two others among the PCB's (1242, 1248, 1254, and 1262) analyzed. If all the major peaks were present for two or more PCB formulations, the peak heights (if analyzed on the Nuclear Chicago 5000) or the peak areas (if analyzed on the Hewlett Packard 5700 with the computing integrator) could be estimated using the distinctive peak heights or areas as a guide. Either alpha or gamma chlordane was distictive from the PCB peaks depending on the column used. Separate standards were run for these, and the nondistinctive peak height/area was found by subtracting the estimated PCB peak height/area from it. DDE, DDD, and DDT heights and areas were found in a similar manner.

At the time the Chester River Study was begun, pure standards of alpha and gamma chlordane were not available, and the standard method utilized technical chlordane (a mixture of the alpha and gamma chlordane isomers and other compounds) as a reference standard (FDA, 1972). As reported at length in the Chester River Study (Clarke, et al., 1972), the method was chosen among a number of unsatisfactory alternatives. Now that pure standards of alpha and gamma chlordane are available, the method of choice seems to be to use them.

In this report, unless specified otherwise, all chlordane and total chlordane values represent the sum of the alpha and gamma isomers. One should bear in mind that these values *are not* directly comparable to values calculated using technical chlordane as the standard. An adjustment to make the values *exactly* comparable can be made only when the samples have been analyzed using the gas chromatographic column packings under identical conditions. An approximate comparison can be made between values calculated as total chlordane (alpha plus gamma) and values calculated as technical chlordane by multiplying the total chlordane values by 2.0.

Frequently an artifact would appear which masked major peaks on one or more columns. In this case, different columns would be used until one was found on which the artifact did not appear.

The quantitation itself used the peak height/area ratio of the major peaks, the total volume of the processed extract, the weight of sample extracted, and the volume of processed extract injected relative to the amount of standard injected. In addition, standard aldrin was injected just prior to each sample to calibrate the response of the gas chromatograph to allow correction for the response of the machine which frequently changed slightly from sample to sample.

6.3 Results

6.3.1 Bottom Sediments

Bottom sediment samples were collected at the beginning of the program on a grid of 52 stations spaced throughout the study area. Figure 6-1 depicts the sample stations, which match the numbers of samples recorded in Volume III, Section 4, Data Summary Reports (BOTM SED PPM). The exact station locations expressed in longitude and latitude are listed under files C414 and C415, Upper Bay Sediment Data Report. Figures 6-2 and 6-3 display the total PCB and DDTR, respectively, found in the bottom sediments at the various stations. The size of circles represents the concentration of the CHC found to make high and low concentrations easily recognized at a glance. (The exact values can be obtained by looking up the particular station of interest in Volume III, Section 4 in File C502 where C506 equals BOTM SED PPM and C503 spans the period October 10 through 16, 1973).

From the data presented in Figure 6-2, one can see that the sediments of Baltimore harbor are quite high in PCB compared with the rest of the bay, except the station at the mouth of the Gunpowder River. Although the range of values found for DDTR in the sediments is much less broad, and Figure 6-3 is much less dramatic than Figure 6-2, the highest values were found in Baltimore harbor and the mouth of the Gunpowder River. Chlordane was not identified in enough samples to make such a figure meaningful.

The results of analyzing 17 additional seasonal bottom sediment samples from Stations 1A, 5A, 7A, 7B, 10B, 11A, and 11C are listed under the appropriate files (where C506 equals BOTM SED PPM) in Volume III, Section 4. Although significant trends are not evident, some of the individual values will be discussed later.

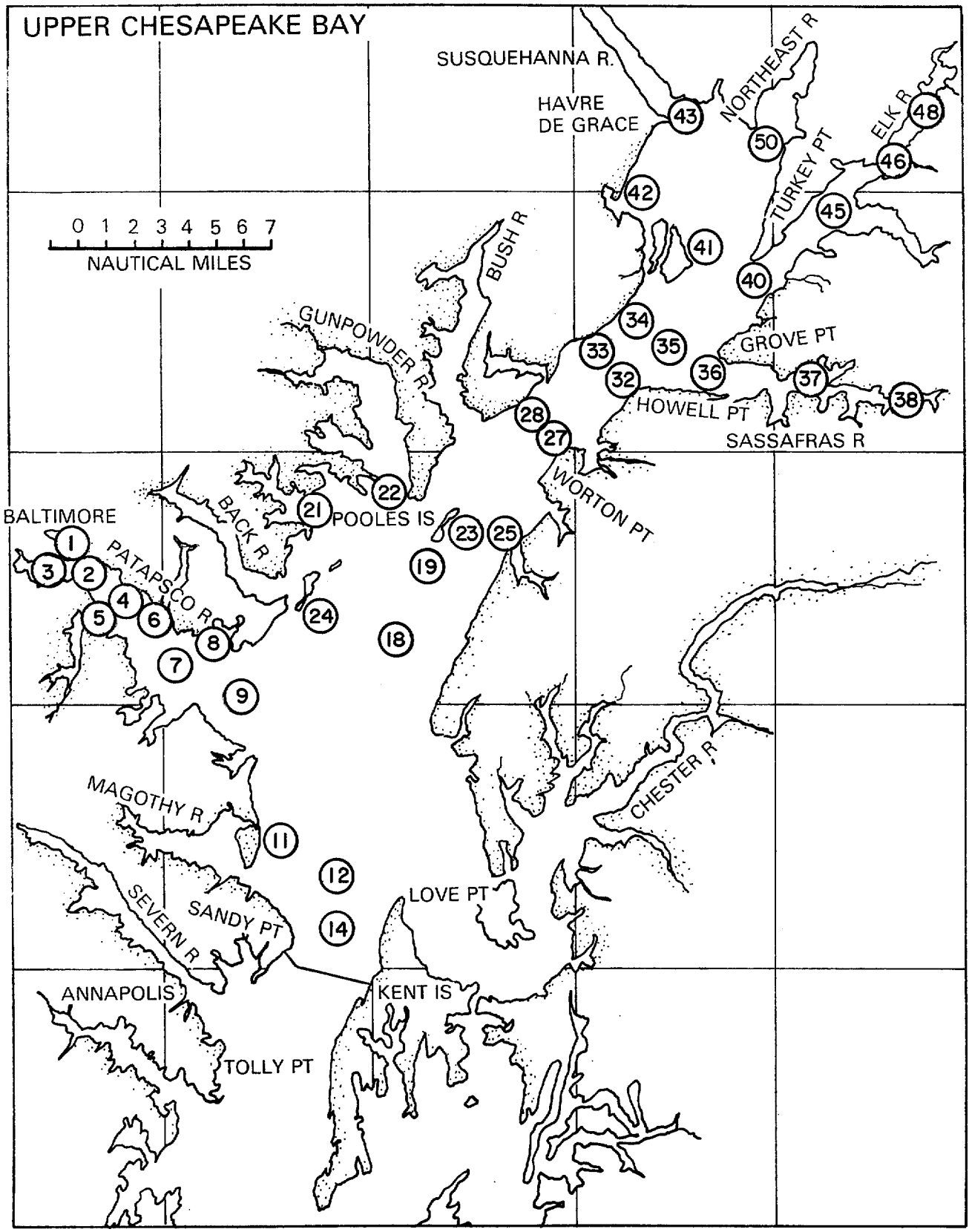

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Figure 6-1. Bottom Sediment Sampling Locations

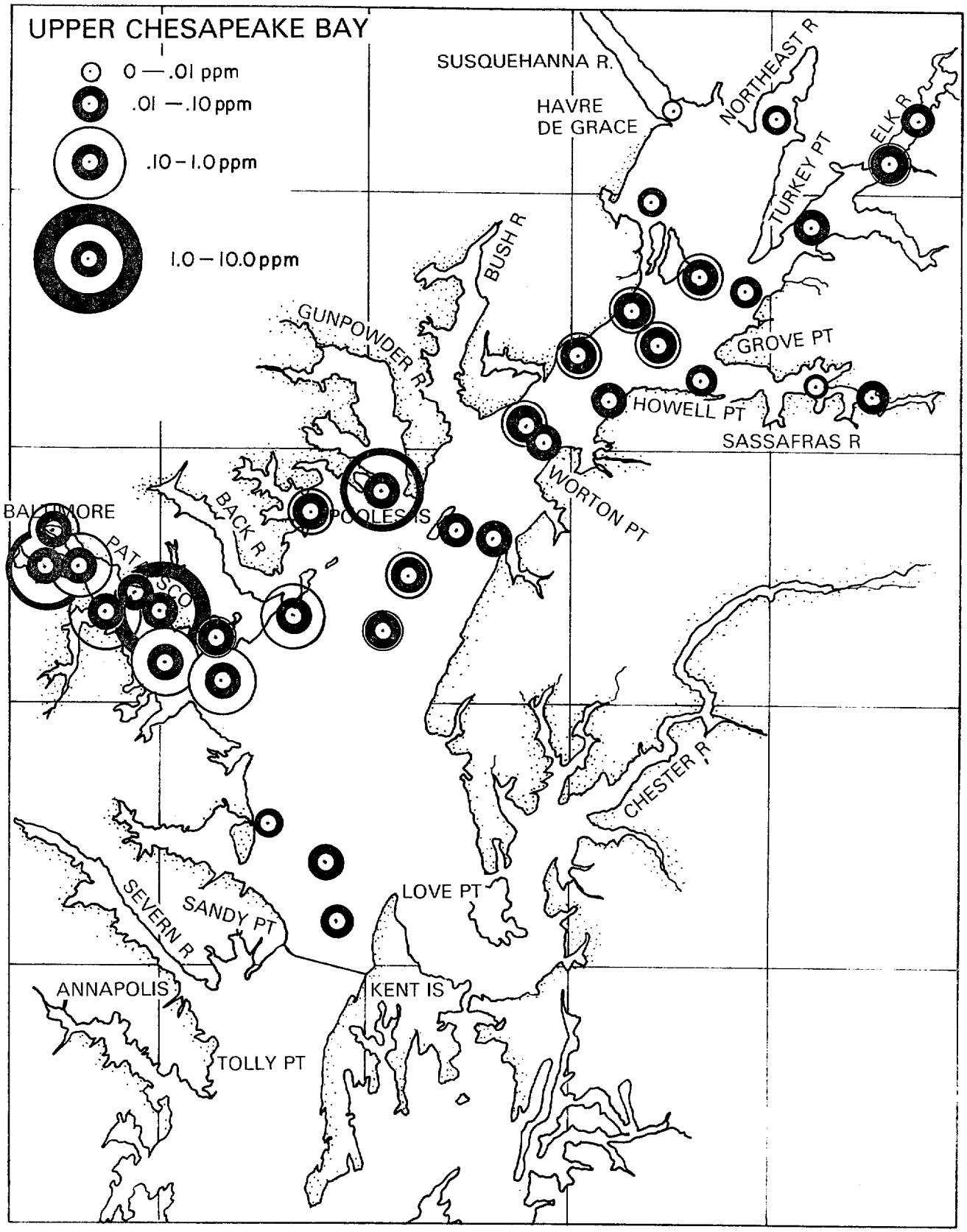

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Figure 6-2. PCB in Bottom Sediments

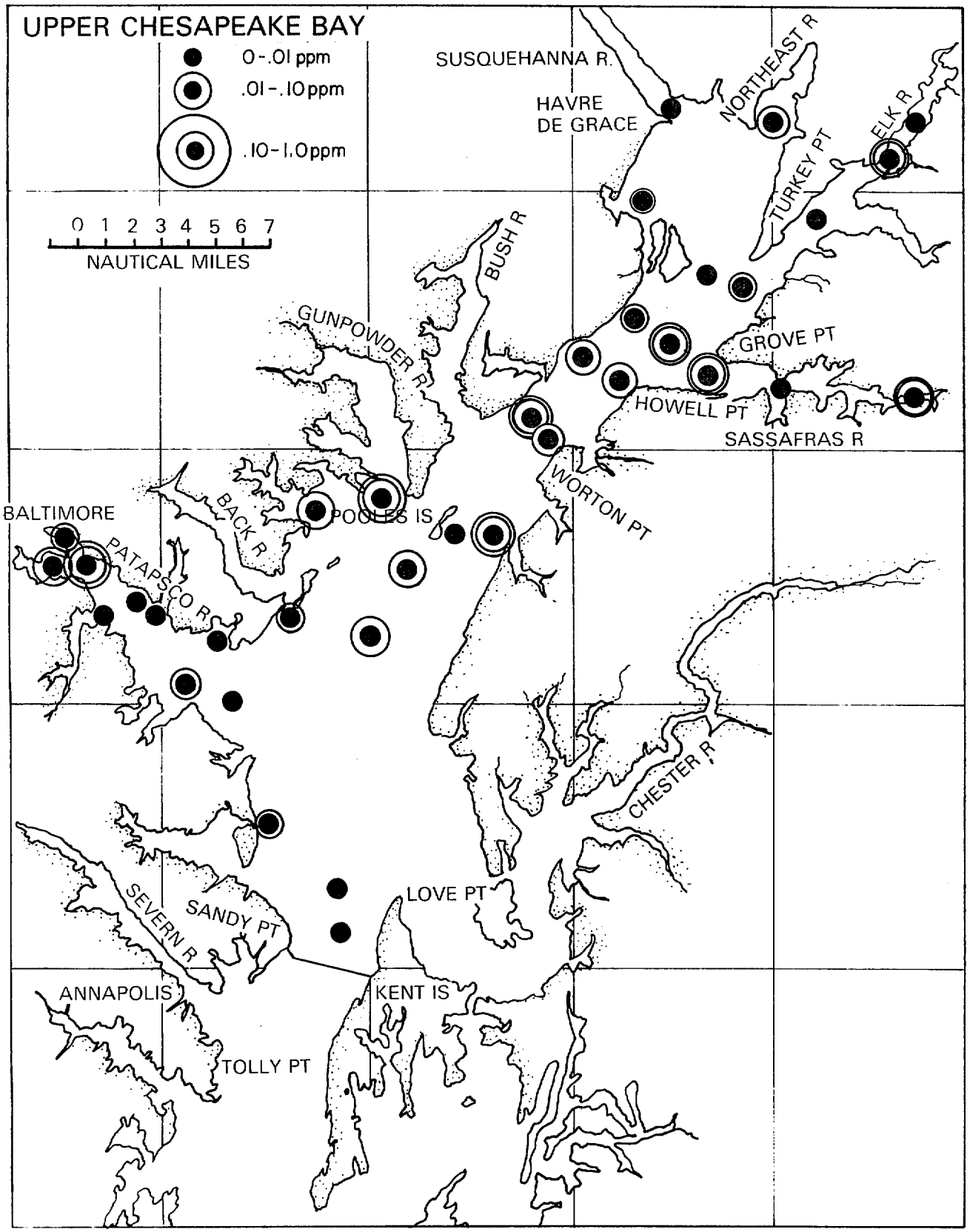

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Figure 6-3. DDTR in Bottom Sediments

6.3.2 Suspended Sediments

Figures 6-4, 6-5, and 6-6 present the average total PCB, average total chlordane, and average DDTR in the suspended sediment and zooplankton samples taken at each station (Figures 2-1 and 5-3). The data have been presented: (1) as micrograms (10^{-6} grams) of CHC found divided by the grams dry weight (suspended sediment samples, Figures 6-4a, 6-5a, and 6-6a) or wet weight (zooplankton samples, Figures 6-4c, 6-5c, and 6-6c) extracted (by definitiion, parts per million, ppm), and (2) as nanograms (10^{-9} grams) CHC found in the sample divided by the liters of bay water filtered to get the sample (by definition, parts per trillion, ppt). The raw data can be found in Volume III, Section 4 by station where file C506 equals S SED DRY PPM, S SED H20 PPT, and PLANK WET PPM and PLANK H20 PPT. Although suspended sediment samples were taken from more than one depth at each station during each sampling period, the averages were computed from the values for all depths at each station combined. More complex bar charts representing the various depths separately did not indicate an obvious depth-related trend and were rather confusing.

Figures 6-4a, 6-5a, and 6-6a show that the average total PCB, total chlordane, and DDTR (sum of the DDE, DDD, DDT residues) were highest in the suspended sediments (on a dry weight basis) taken from Baltimore harbor (Station 7A). When the data are expressed as parts per trillion of CHC in the water on suspended sediment (Figures 6-4b, 6-5b, and 6-6b), a somewhat different pattern appears. The average PCB, chlordane, and DDTR values generally show a decreasing trend down the bay with the harbor station not as high (in the case of PCB) or about the same as the upper stations (in the case of chlordane and DDTR). This decreasing trend is not nearly as pronounced in the chlordane and DDTR figures as it is in the PCB figure.

Figures 6-7, 6-8, and 6-9 present the CHC data for suspended sediments and zooplankton averaged by data collected. As in the previous set of figures, the data are presented both as parts per million based upon the weight of sample extracted, and as parts per trillion based upon the volume of water filtered to yield the sample extracted.

Figures 6-7a and b seem to indicate that the total PCB concentration on the suspended sediments and in the water on suspended sediment decreased somewhat from December through July. However, this apparent decrease probably is not real for several reasons. When the individual data points are plotted by collection date at each station and the resultant 18 bar charts examined, no such baywide seasonal trend is evident. In addition, the June and July data sets were quite limited in the number of samples analyzed, and the averages were not composed of values from all of the stations. (In particular, the harbor and upper stations were missing.) Figures 6-8a and b suggest a general increase in the chlordane from December through July, and a closer examination of the data from which the averages were derived supports the existence of a seasonal trend.

Figures 6-9a and b indicate a general increase in the DDTR concentration on the suspended sediment and in the water on suspended sediment from December through July. The detailed bar charts support the generally increasing trend evident in the average values.

6.3.3 Zooplankton

The average PCB, chlordane, and DDT residues found in the zooplankton at each of the stations sampled are shown in Figure 6-4c, 6-5c, and 6-6c respectively. The values are expressed as micrograms of CHC found divided by the grams wet weight of zooplankton extracted (ppm). The spatial distribution of the average PCB in zooplankton (by station) shows a dramatically pronounced trend—decreasing down the bay from very high values at the upper stations. The average PCB values at Stations 1A and 3B are about ten times the values at the lower stations. An examination of the data from which the average values were derived support this trend (although, as seen in Figure 6-7c, the values vary over a broad range from one collection period to the next). The DDTR values follow a similar trend, except in this case, Station 1A stands alone much higher, the rest of the stations showing generally decreasing values down the bay. This pattern is the result of two unusual samples from Station 1A being included in the average values. The Station 1A zooplankton samples had DDTR values of 4.2 ppm in June and 3.9 ppm in September. These unusually high DDTR values resulted almost entirely from the presence of DDD in the samples. (More will be said about these anomalous samples later.)

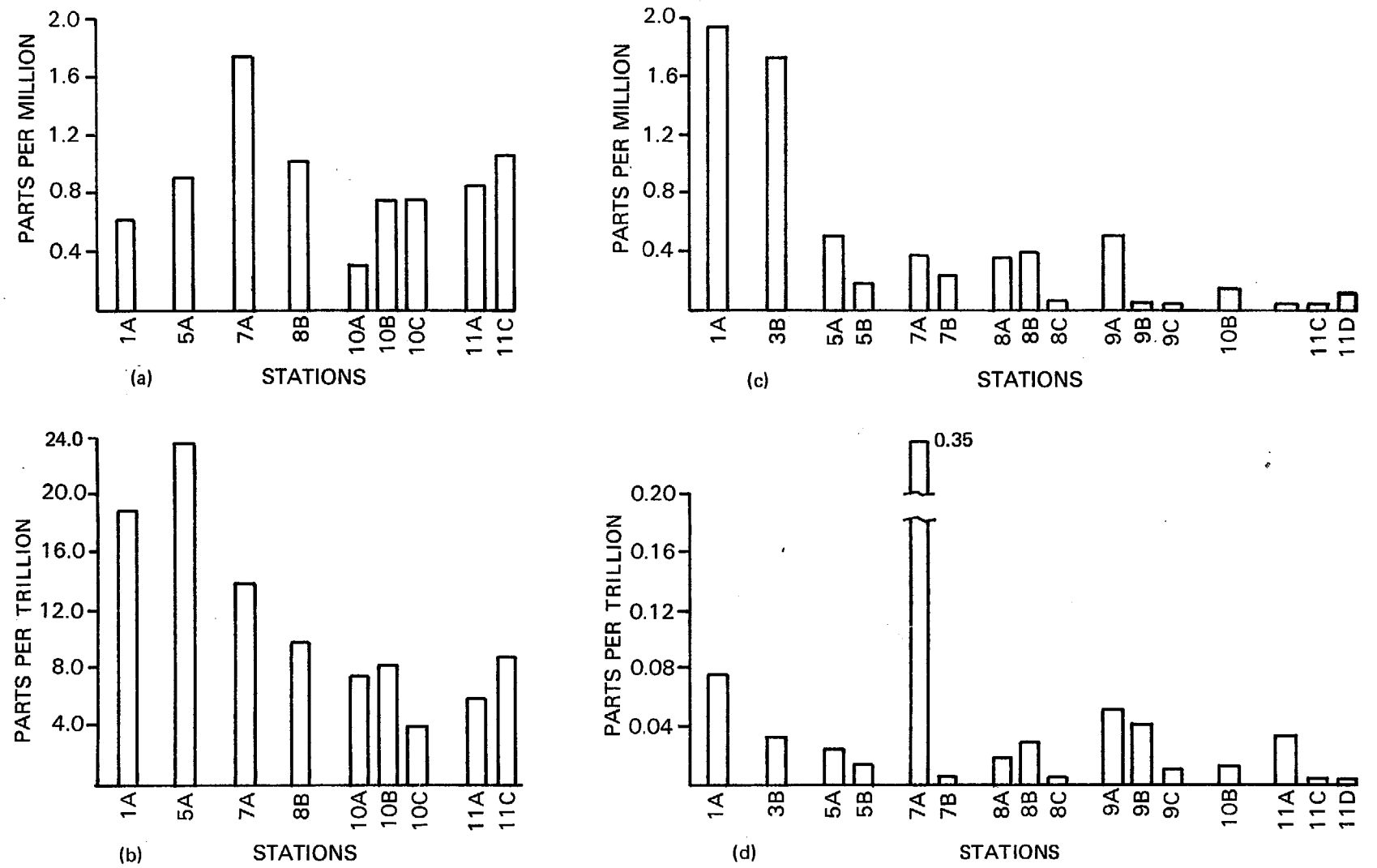

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Figure 6-4. Average PCB Concentrations by Stations (a) on Suspended Sediment, Dry, (b) in Water on Suspended Sediment, (c) on Plankton, Wet, (d) in Water on Plankton

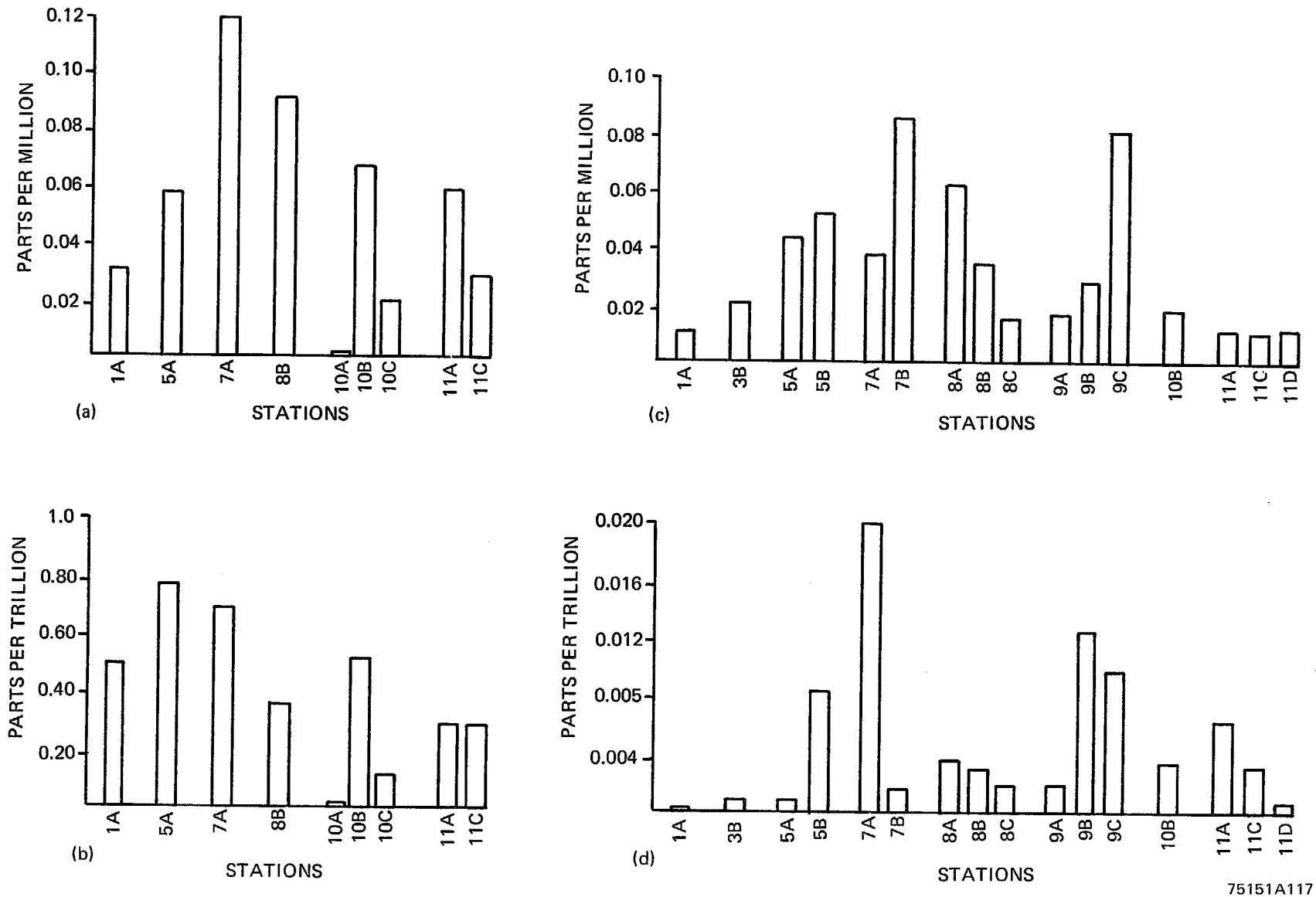

Figure 6-5. Average Chlordane Concentrations by Station (a) on Suspended Sediment, Dry (b) in Water on Suspended Sediment (c) on Plankton, Wet, (d) in Water on Plankton

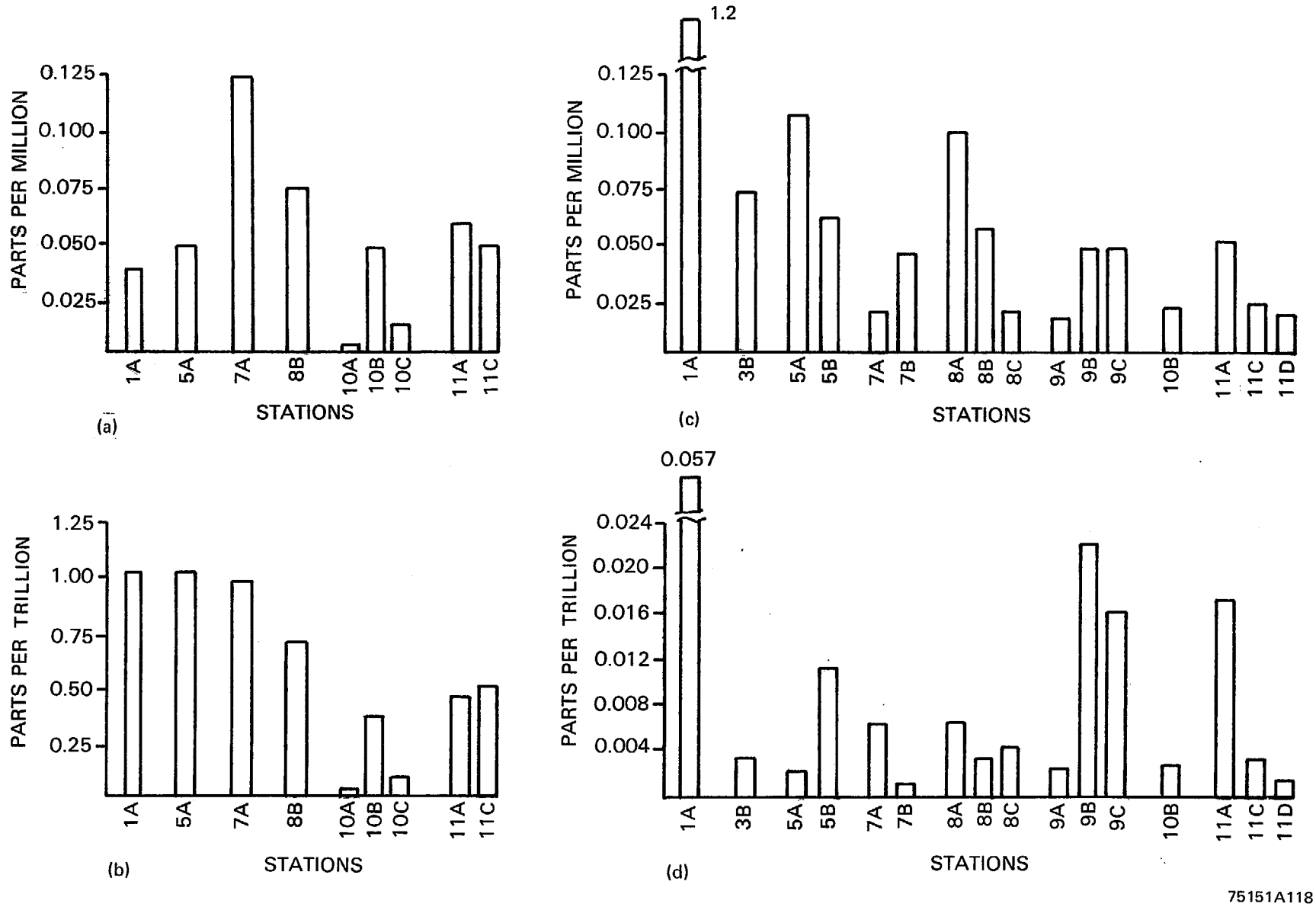

Figure 6-6. Average DDTR Concentrations by Station (a) on Suspended Sediment, Dry (b) in Water on Suspended Sediment (c) on Plankton, Wet (d) in Water on Plankton

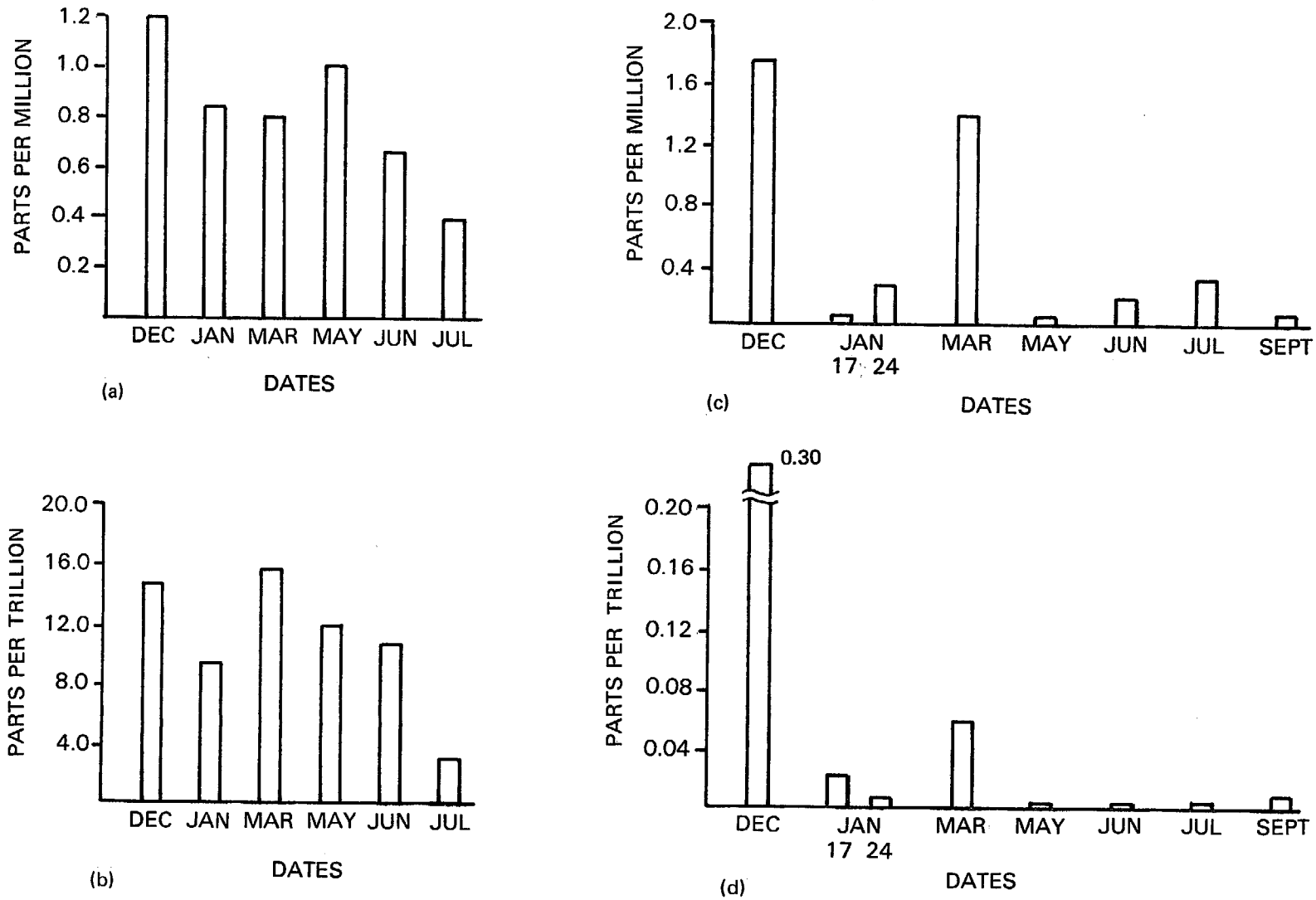

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Figure 6-7. Average PCB Concentrations by Date (a) on Suspended Sediment, Dry (b) in Water on Suspended Sediment (c) on Plankton, Wet, (d) in Water on Plankton

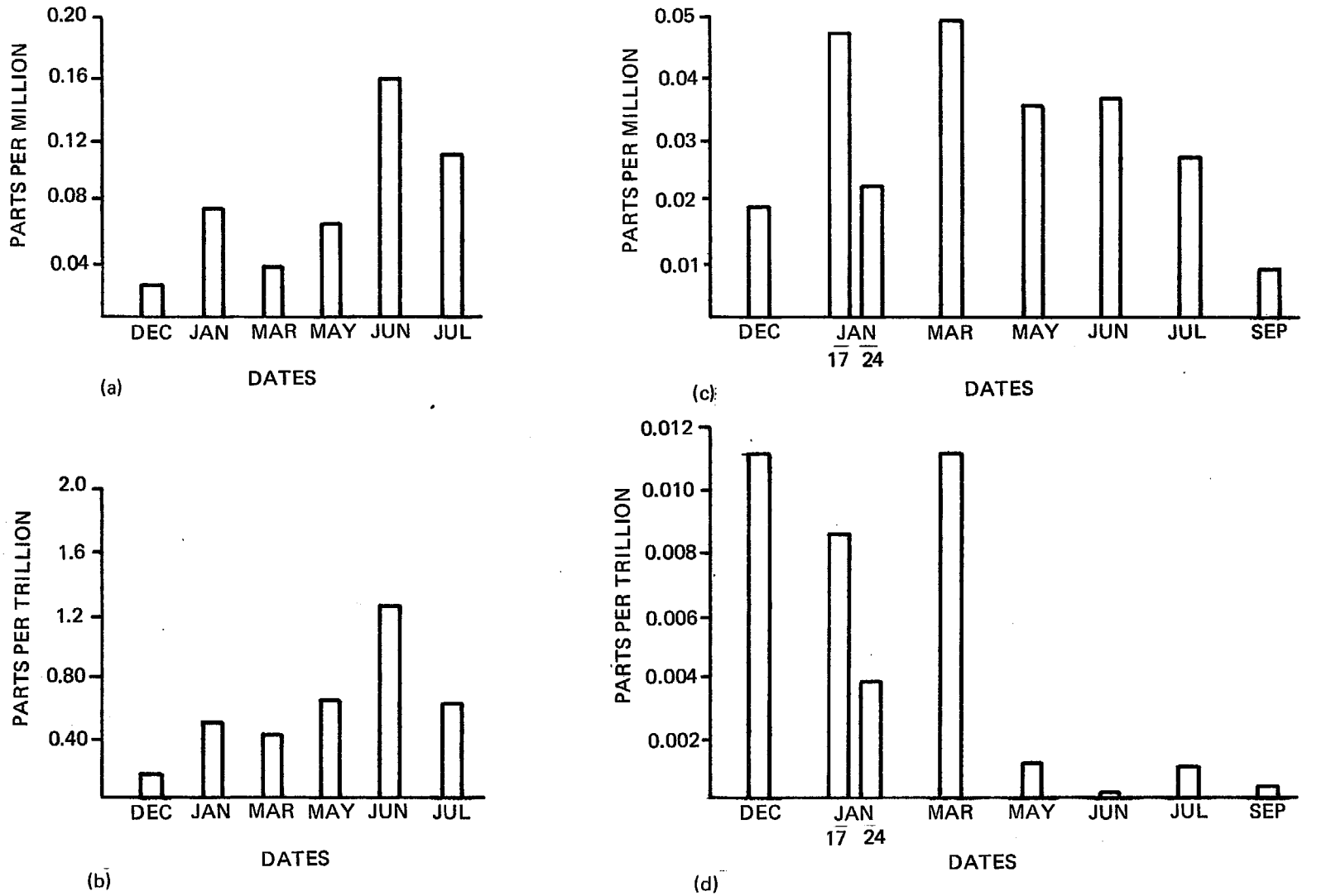

Figure 6-8. Average Chlordane Concentrations by Date (a) on Suspended Sediments Dry (b) in Water on Suspended Sediment (c) on Plankton, Wet (d) in Water on Plankton

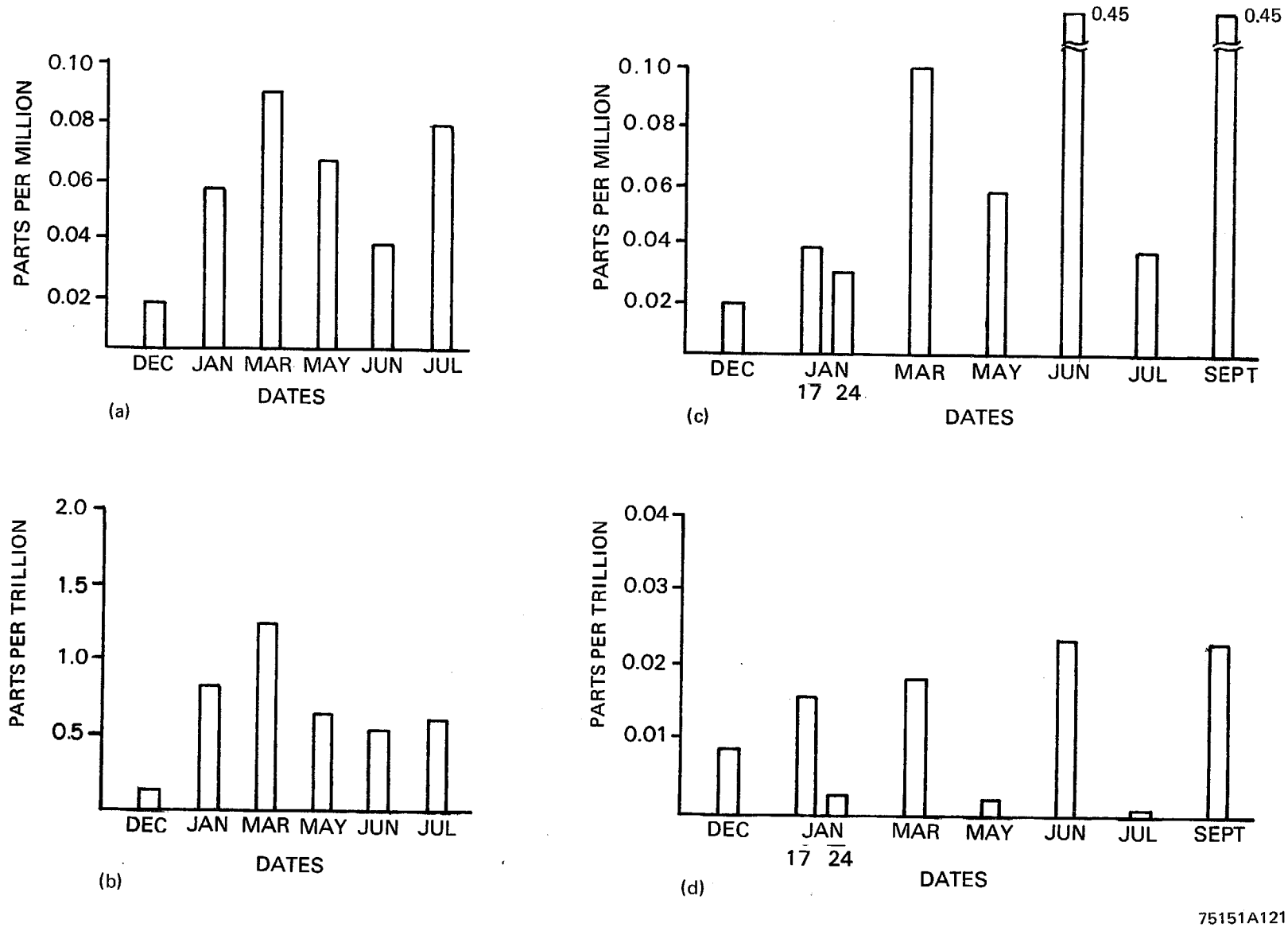

Figure 6-9. Average DDTR Concentrations by Date (a) on Suspended Sediment, Dry (b) in Water on Suspended Sediment (c) onPlankton, Wet (d) in Water on Plankton

Figure 6-5c shows apparent highs in the chlordane values at Baltimore harbor Station 7B and Chester River Station 9C. The individual data do indicate that the Station 7B samples were high; however, the value for Station 9C is derived from a single analysis and may not be a representative value.

Figures 6-4d, 6-5d, and 6-6d present the respective average values as parts per trillion by station for PCB, chlordane, and DDTR based upon the volume of water filtered to obtain the sample extracted. The PCB values now present quite a different picture compared to those calculated using the wet weight of the sample. In this case. harbor Station 7A has by far the highest average PCB level, due entirely to a value of 1.3 ppt at this station in December. In fact, the values do not display a pronounced spatial trend when examined as individual bar charts segregated by collection date.

When the chlordane values are expressed as parts per trillion in the water in or on zooplankton, the harbor still appears high (in this case, Station 7A). An examination of the individual plots support the average picture presented in Figure 6-5d; although, being single values, the numbers at Stations 9B and 9C may not be representative of average conditions.

Figure 6-6d shows that the average DDTR pattern changes slightly when calculated as ppt DDTR in the water in zooplankton. The overwhelming high at the uppermost Station 1A remains, while the general trend of decreasing values proceeding down the bay appears to have become somewhat reversed. A detailed examination of the individual bar charts, while generally supporting the increasing trend down the bay, reveals that the overwhelmingly high average value at the head of the bay (Station 1A) results from two of seven samples (June and September) being very high in DDD.

Figures 6-7c and d presenting the average PCB values arranged by collection period show that the average PCB values were high during December and March when expressed both as parts per million based upon the wet weight of zooplankton extracted and as parts per trillion based upon the volume of water filtered to get the sample. An examination of 16 individual bar charts representing the data plotted by station and by date indicates that these two charts of the average values fairly present the trends evident in the data.

Figure 6-8c and d both appear to give a good representation of the trends apparent in the individual plots. The chlordane concentration in the zooplankton (ppm, wet weight) appears fairly constant during the sampling period except for a slight low in September. The concentration of chlordane in the water column in zooplankton (ppt), however, shows highs in three months—December, January, and March.

The average DDTR bar charts (Figures 6-9c and d) do not represent the trends in the actual data very well because of two previously mentioned samples with exceptionally high levels of DDD. Were it not for these two anomalously high samples at Station 1A (June and September), the overall monthly averages would show a pattern very similar to that seen for chlordane—a slight high in March in the concentration of DDTR in zooplankton (ppm) and high in December, January, and March in the water column concentration of DDTR (ppt in the water on zooplankton).

6.3.4 Benthic Organisms

Samples of benthic organisms were collected and analyzed to provide an estimation of the movement of chlorinated hydrocarbons into this biological community. Table 6-1 presents the results of the analyses of benthic samples.

6.3.5 Laboratory and In Situ PCB Exposure Experiments

Results from the analyses performed in support of dosing experiments using the PCB formulation Aroclor® 1254 in the laboratory and *in situ* are presented and discussed in Chapter 2, Marine Biology.

TABLE 6-1. CHLORINATED HYDROCARBONS IN BENTHIC ORGANISMS IN THE UPPER CHESAPEAKE BAY (ppm)

Organism	Station	Collection Date	Total PCB	Total Chlordane	DDTR
SHELLFISH:					
Rangia cuneata	4A	9/20/74	0.081	0.0092	0.026
Rangia cuneata	5C	5/03/74	0.0090	0.0040	0.018
Rangia cuneata	5C	8/02/74	0.12	0.010	0.055
Rangia cuneata	6A	3/22/74	0.031	0.016	0.027
Rangia cuneata	6A	5/03/74	0.087	0.054	0.23
Rangia cuneata	6A	8/02/74	0.089	0.064	0.019
Rangia cuneata	6A	9/20/74	0.058	0.0066	0.027
Rangia cuneata	6B	3/22/74	0.051	0.025	0.032
Rangia cuneata	6C	3/22/74	0.031	0.016	0.0040
Rangia cuneata	6C	5/03/74	0.0020	0.0002	0.00020
Rangia cuneata	6C	8/02/74	0.045	0.0059	0.024
Rangia cuneata	6C	9/20/74	0.093	0.0042	0.029
Rangia cuneata	8A	3/22/74	0.094	0.017	0.047
Rangia cuneata	8A	8/02/74	0.074	0.0017	0.037
Rangia cuneata	8A	8/26/74	0.15	0.033	0.056
Rangia cuneata	8A	9/20/74	0.0084	0.012	0.023
Rangia cuneata	8B	3/20/74	0.043	0.011	0.019
Rangia cuneata	10A	5/03/74	0.064	0.0094	0.054
Rangia cuneata	10A	9/20/74	0.029	0.051	0.028
Crassostrea virginica	8B	1/25/74	0.021	0	0.014
Crassostrea virginica	10A	1/25/74	0.031	0.0098	0.014
Crassostrea virginica	10A	3/22/74	0.054	0	0.034
Crassostrea virginica	10A	8/02/74	0.015	0.0099	0.017
Crassostrea virginica	11A	3/20/74	0	0.017	0.025
Brachiodontis recurvus	8A	3/22/74	0.046	0.016	0.015
Macoma sp	10A	8/02/74	0.029	0.0099	0.031
Shellfish Averages			0.052	0.016	0.035
CRABS:					
Callinectes sapidus	10A	3/02/74	1.2	0.15	0.72

6.3.6 Other Samples

6.3.6.1 Rain Water-Ground Water-Storm Water

The chlorinated hydrocarbon data for the rain water, storm water, and ground water samples are presented in Tables 5-5, 5-6, and 5-7, respectively and will be discussed later in this chapter. Substantial quantities of PCB and chlordane were found in the rain water samples from the collection station at Sollers Point as well as in the storm water samples. These compounds were also present in the air samples. An important point about the rain water samples is that two pesticides (toxaphene and benzene hexachloride) which have not been found in abundance elsewhere in the study were found in substantial quantities in some of the rain water samples.

6.3.6.2 Conowingo Dam Samples

It became apparent as the analysis of the initial samples of suspended sediments and plankton got underway, that Station 1A was an area of high PCB concentrations. At the recommendation of the Board of Scientific direction, an attempt was made to gather data to resolve the question as to the origin of the material. (Was it being added to the system near Station 1A, or was it actually coming down the Susquehanna River?) Table 6-2 presents the data which were obtained to address this question.

Suspended sediments were collected nearly simultaneously at Havre de Grace (Station 1A) and at the tailrace of the Conowingo Dam (Station 1B) on two occasions. Attempts were made to gather samples on several other occasions, but fate seemed to be against this particular effort—either one sample or the other was lost or was not taken due to oversight or misadventure.

6.4 Discussion

Table 6-3 summarizes the chlorinated hydrocarbon levels found in the various types of samples collected in the upper Chesapeake Bay. Although, the standard deviations indicate that the ranges of values are quite broad, the values are striking when compared to similar data from the Chester River Study—Table 6-4. The concentrations in bottom sediments are two to three times higher in the upper Chesapeake Bay than in the Chester River.* From Table 6-1, one can see that the PCB, chlordane, and DDTR in shellfish are about the same. CHC's in the single crab analyzed were more than 20 times higher than the average values for crabs from the Chester River. Although, the values from a single organism would usually be considered to have little statistical significance, the fact that the PCB, chlordane, and DDTR values were more than ten times higher than the highest values observed in the Chester River crabs suggest a significant difference. These comparisons will be discussed subsequently in this section.

By comparing the bottom sediment (dry) data with the suspended sediment (dry) data, one can see that the PCB and chlordane concentration are four to ten times higher in the suspended sediments. The higher values in the suspended sediment probably occur for one, or most likely, both of the following reasons. The average grain-size of the suspended sediments is much smaller than that of the bottom sediments, resulting in a greater surface area for adsorption per unit weight as discussed in detail in Chapter 4, Estuarine Sedimentology. Although, the suspended sediments are primarily inorganic materials, the phytoplankton, which were included in these samples, probably bio-concentrated chlorinated hydrocarbons.

If one accepts the above discussion, he is left with the dilemma that the DDTR does not appear higher in the suspended sediments as do the PCB and chlordane. But all uses of DDT were banned in the United States at the end of 1972 (CEQ, 1972). If this ban had the effect of decreasing the DDT levels flowing into the Chesapeake Bay, the concentrations of DDT residues in the suspended sediments would be less relative to those in the bottom sediments. This is because the bottom sediment samples consist of materials deposited during the sampling year and during previous years as well, while the suspended sediment samples consist primarily of materials that entered the bay during the sampling year. While

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*As pointed out in discussing analytical methods in Section 6.2.2.2, to compare the total chlordane values presented in this report to the technical chlordane numbers recorded in the Chester River Study, the total chlordane values must be multiplied by 2.0.

TABLE 6-2. CHLORINATED HYDROCARBONS IN SUSPENDED SEDIMENTS FROM HAVRE DE GRACE (STATION 1A) AND CONOWINGO DAM (STATION 1B)

STATION	DATE	TOTAL PCB*	CHLORDANE*	DDE*	DDE*	DDT*
1A (surface)	6/13/74	0.77	0.30	0.029	0.20	--
1A (bottom)	6/13/74	0.83	0.19	0.10	0.73	0.53
1B	6/13/74	3.6	0.29	0.052	0.72	--
1A	9/20/74	9.3	0.21	--	0.23	--
1B	9/20/74	10.	0.077	--	0.052	0.10

*All of the values are expressed as nanograms of material extracted from suspended sediment divided by the volume of water (in liters) filtered to obtain the suspended sediment.

TABLE 6-3. AVERAGE CHLORINATED HYDROCARBONS FOUND IN THE UPPER CHESAPEAKE BAY
(Standard Deviations are in Parentheses)

Sample Type	Number of Samples	Total PCB	Total Chlordane	Total DDT
Shellfish (Wet, ppm)*	26	0.052 (0.037)	0.016 (0.017)	0.035 (0.041)
Plankton (Wet, ppm)	70	0.50 (1.4)	0.041 (0.032)	0.16 (0.68)
Suspended Sediment (Dry, ppm)**	66	0.92 (0.87)	0.061 (0.086)	0.057 (0.066)
Bottom Sediment (Dry, ppm)	54	0.28 (0.57)	0.0052 (0.014)	0.051 (0.067)
Plankton (H_2O, ppt)***	69	0.042 (0.164)	0.0038 (0.0083)	0.010 (0.035)
Suspended Sediment (H_2O, ppt)	68	12 (14)	0.53 (0.88)	0.78 (1.5)

*The values are expressed as micrograms CHC found per gram wet weight of material extracted.
**The values are expressed as micrograms CHC found per gram dry weight of material extracted.
***The values are expressed as nanograms of CHC found per liter of water filtered to collect the material extracted.

TABLE 6-4. TYPES AND LEVELS OF CHLORINATED HYDROCARBONS FOUND IN THE CHESTER RIVER (CLARKE, et al., 1972)

Sample	PCB'S	Chlordane	DDT (Total)
OYSTERS:			
Average (ppm)	0.055	0.036	0.043
Range (ppm)	0.016 to 0.250	0.009 to 0.160	0.0 to 0.150
SOFT-SHELLED CLAMS:			
Average (ppm)	0.058	0.014	0.021
Range (ppm)	0.013 to 0.180	0.0 to 0.038	0.0041 to 0.130
FISH:			
Average (ppm)	0.185	0.074	0.134
Range (ppm)	0.002 to 0.570	0.034 to 0.100	0.050 to 0.260
CRABS:			
Average (ppm)	0.020	0.014	0.033
Range (ppm)	0.0004 to 0.051	0.003 to 0.024	0.018 to 0.063
SEDIMENTS:			
Average (ppm)	0.087	0.0052	0.016
Range (ppm)	0.0 to 0.310	0.0002 to 0.014	0.0 to 0.063

this explanation is plausible, it seems somewhat suspicious that samples were taken fortuitously at just the right time to yield exactly the same DDT residue levels in both the suspended sediments and the bottom sediments.

The data in Table 6-3 also show that the CHC's are bio-concentrated in passing from the suspended sediment into the zooplankton. To evaluate the magnification, one must first convert the plankton (wet) values to plankton (dry) to allow comparison with the suspended sediment (dry) values. From Table 2-5 in Chapter 2, one finds that the December to September ratios of plankton dry weight to plankton wet weight range from 0.11 to 0.17. If one conservatively uses 0.10, he multiplies all of the plankton (wet) values by ten to convert to plankton (dry) and then calculates the following bio-concentrations as the CHC's pass from the suspended sediments to the zooplankton: PCB, 5.4; chlordane, 6.7; and DDTR, 28. The DDTR value seems unreasonably high, and it probably is. If the two anomalously high DDTR values mentioned earlier are excluded and the average is computed from the other 68 values, the average DDTR in plankton (wet) becomes 0.045, leading to a magnification of 7.9—a value more compatible with the others.

One also could calculate approximate bio-magnification values from the shellfish data. The concentrating ability of shellfish usually is expressed as the level accumulated in the shellfish tissue (wet weight) divided by the exposure concentration in the water. Using the values for CHC in the water column on suspended sediment, the shellfish concentration factors are approximately: PCB, 4,000; chlordane, 30,000; and DDTR, 45,000. These values should be considered rough estimates, because the average values used for CHC's in the water column probably do not represent accurately the values at the water-bottom sediment interface inhabited by these shellfish.

The standard deviation values given in Table 6-3 emphasize the fact that the range of CHC values observed within any particular sample type was very broad. Upon examining the bar charts summarizing the data for CHC's on suspended sediment and zooplankton, one can see that the values vary greatly from one station to the next during the same collection period or at the same station from one collection period to the next. Apparently these values fluctuate rapidly enough (temporally and spatially) that only the unusually large events in terms of time and/or space will be observed at enough points to describe a trend when the sampling is limited to a fairly small number of samples, as in this program. The multidisciplinary approach of this program, however, circumvents this problem somewhat by providing many different types of data which are comparable in space and time. As will be seen below, these data then can be examined for interrelationships relevant to the understanding of the dynamics of chlorinated hydrocarbon movements in the upper Chesapeake Bay.

The CHC data for suspended sediments and zooplankton are expressed in two ways, as mentioned earlier, but further amplification should be made. Environmental residues of chlorinated hydrocarbons (chlorinated pesticides and polychlorinated biphenyls) usually are reported as parts per million (ppm) dry weight (or wet weight) based upon the dry weight (or wet weight) of sample which was extracted for the analysis. If the levels are very low, parts per billion (ppb) or parts per trillion (ppt) also are used. The data then represent the concentration of CHC's present in that sample. In the consideration of transport of CHC's (rates, routes, etc.) the *amount* of CHC being transported by a water movement frequently is of more interest than the *concentration* of the CHC's on the suspended sediments in the water. Therefore, in addition to the data being presented in the usual fashion, the zooplankton and suspended sediment CHC data are presented as nanograms (10^{-9} grams) of CHC on zooplankton or suspended sediment per liter of water (by definition, ppt). These values represent the amount of CHC associated with the suspended sediments or the zooplankton contained in a one liter sample of bay water. In a sense, the numbers can be compared directly because they have been normalized to a unit volume of water. For instance, in referring to Table 6-4, one finds the average PCB values in the bay water on suspended sediment and zooplankton to be 12 ppt and 0.042 ppt, respectively. On the average, a given volume of bay water will have about 300 times as much PCB in the suspended sediment fraction as in the zooplankton fraction.

Figure 6-10 is a log-log plot of total CHC* concentration in the water column on suspended sediment (ppt) versus the concentration of suspended sediment in the water (milligrams per liter). Although the data points are widely scattered, the data could best be enclosed in a oblong field with an upward tilt

*The sum of PCB, chlordane, and DDTR.

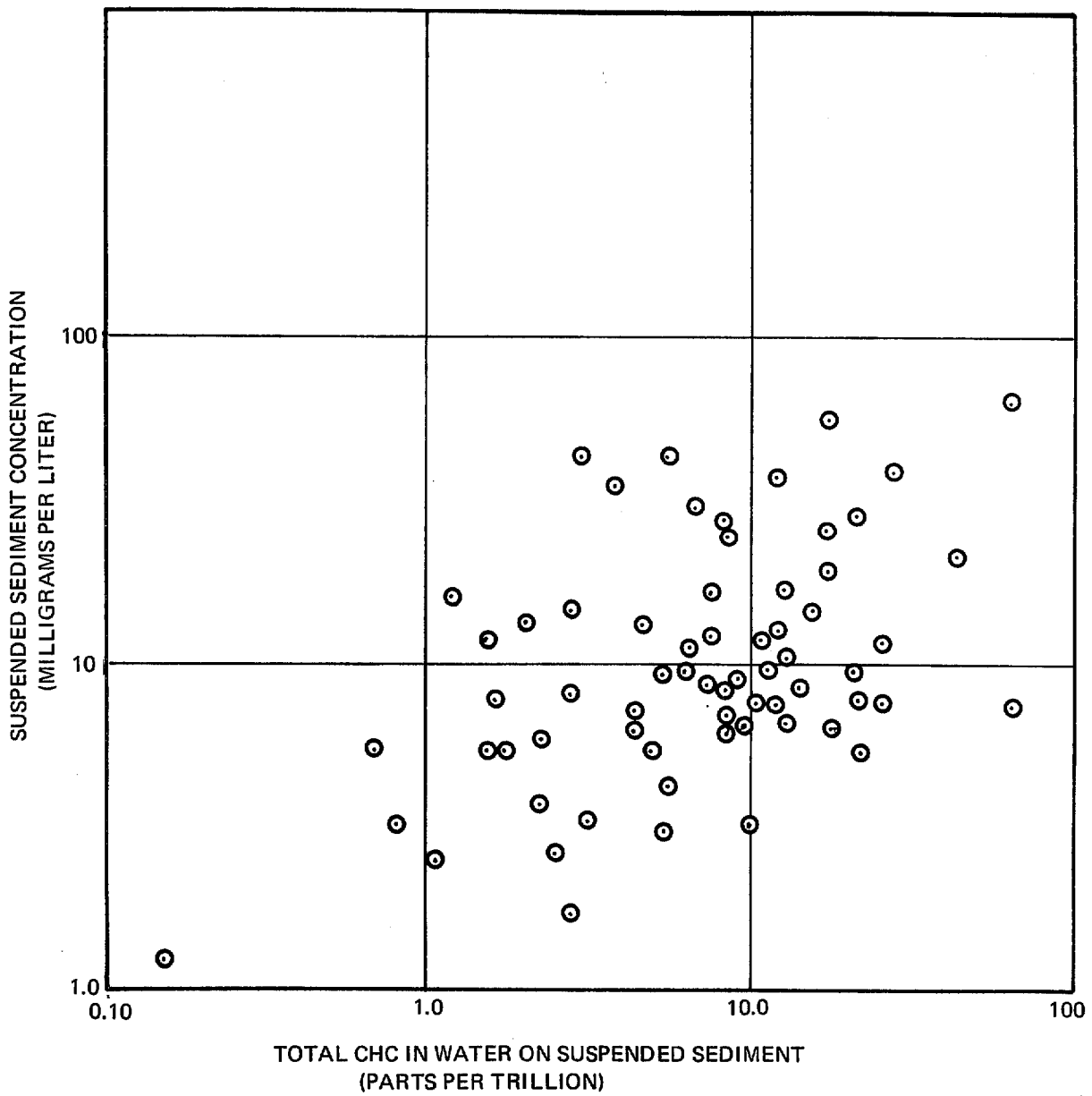

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Figure 6-10. Concentration of Chlorinated Hydrocarbons in the Water Column on Suspended Sediments versus the Concentration of Suspended Sediments

to its long axis. This indicates a positive relationship between the parameters plotted (if the parameters were unrelated—that is, varied independently, the long axis of the oblong would be either vertical or horizontal). The correlation obviously is not followed closely by all of the data, but in general, it indicates that the bay water samples which had high suspended sediment concentrations also had high levels of CHC's in the water on suspended sediments. If all suspended sediments in the water contained the same amounts of CHC's (ppm, dry weight), a perfect correlation would have been observed in Figure 6-10. The variations in the concentrations of CHC's on suspended sediments are responsible for the scattering of the data.

Figure 6-11 is a log-log plot of total CHC's in the water on zooplankton (ppt) versus the zooplankton biomass in the water (milligrams per cubic meter). Although the data are somewhat scattered, there is an obvious positive relationship—as the zooplankton population in the water increases (increasing biomass), the amount of CHC's in the water on zooplankton increases. The existence of this relationship establishes that there is movement of CHC's into the aquatic food chains which include the zooplankton community. The fact that the zooplankton population contains only a small fraction of the CHC's present in the water column, considered with the relationship observed in Figure 6-11, suggests that the movement of CHC's into the biological system (via zooplankton) from the non-biological system (suspended sediments) is not influenced by changes in the concentration of suspended sediment but is regulated by whatever regulates the zooplankton population. One could visualize the suspended sediments in the water column as being a *reservoir* of CHC's which flow into the biological system when zooplankton blooms occur.

Obviously, this concept is an over simplification of a very complex system. More likely, the phytoplankton are important in the transport of CHC's from the non-biological reservoir into the zooplankton food-chain. Unfortunately, the sampling procedure for gathering suspended sediments involved pumping the water through a filter, so the phytoplankton were included in the fraction called *suspended sediments.* To overcome this limitation, it had been hoped to estimate the influence of changes in the phytoplankton population through the use of measurements of the chlorophyll level in the water column. Figure 6-12 presents a log-log plot of the zooplankton biomass versus the chlorophyll-a concentration in the water column. These parameters appear to be independent variables. A similar plot (not shown) of the chlorophyll-a concentration versus the concentration of CHC's in the water column on zooplankton yielded a very similar result. If the phytoplankton have a direct role in the pathway of CHC's into the zooplankton community, the chlorophyll-a data here do not indicate it.

Whatever the details of the pathway for CHC's from the suspended sediment reservoir into the zooplankton, it seems that only a very small percentage of the CHC's in the water column are associated with the zooplankton (average of 12 ppt PCB in the water column on suspended sediment versus 0.042 on zooplankton). However, to quantify the rate of movement of the CHC's via this pathway one would need information about turnover rates in the plankton community. Regarding resource management, one should consider that a change in bay conditions which would increase the zooplankton population would probably have the effect of increasing the flow of CHC's from the suspended sediment reservoir into the biological system. This could create adverse consequences as all aquatic species of commerical interest at some time in their life cycle make up a part of the zooplankton community.

The filtering of bay water by shellfish represents another direct route for th movement of CHC's from the suspended sediment reservoir into the biological system. Although one cannot directly assess the magnitude of this pathway in terms of mass-flow of CHC's, the data in Table 6-1 indicate that substantial amounts of the CHC's present in the reservoir are accumulating in the tissues of shellfish in the upper Chesapeake Bay. These few data points probably do not accurately describe the CHC levels in shellfish in the upper bay, but they do indicate that *Rangia cuneata* accumulates chlorinated hydrocarbons, and they give some idea at least of the minimum range of values.

None of the CHC levels in shellfish approached the levels which the U. S. Food and Drug Administration has suggested as making edible tissues unfit for human consumption (5.0 ppm for PCB and DDTR; 0.30 ppm for chlordane). Nevertheless, all pathways into organisms which are routinely gathered for human consumption warrant further study because of the concern about potential detrimental effects of these chlorinated hydrocarbons. In July 1975, the U. S. Environmental Protection Agency

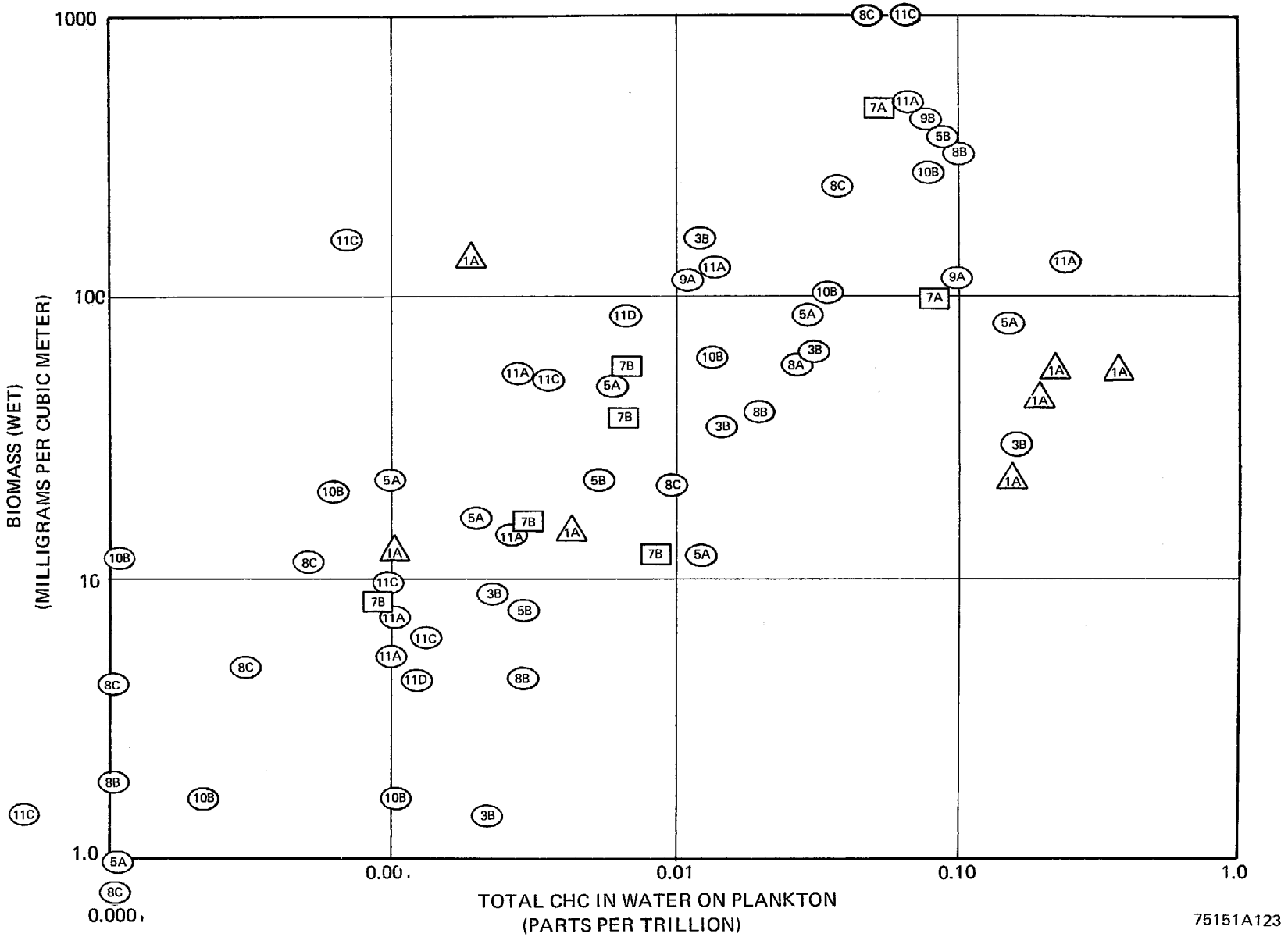

Figure 6-11. Chlorinated Hydrocarbons in the Water on Zooplankton (ppt) versus Zooplankton Biomass in the Water (mg/m^3)

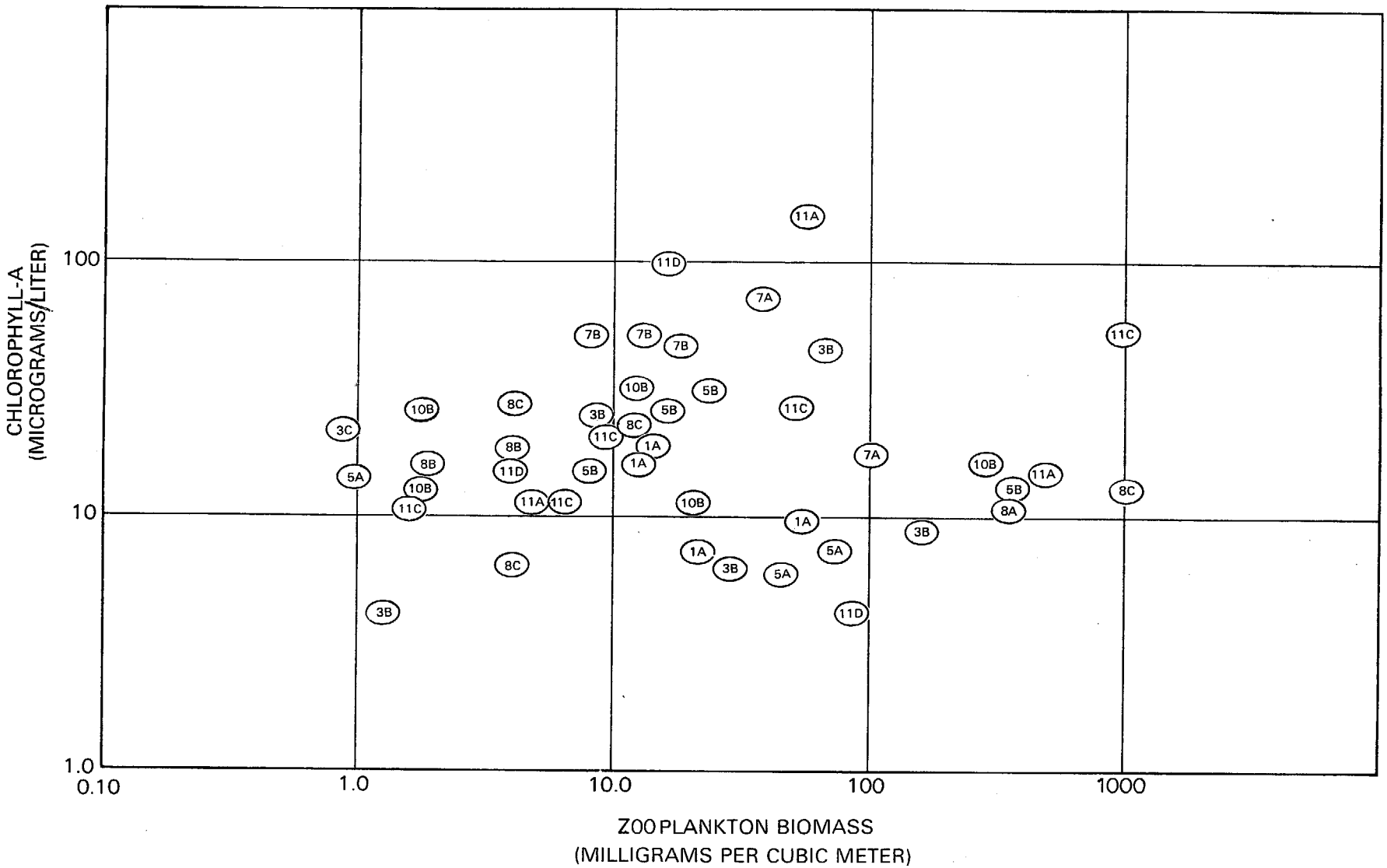

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Figure 6-12. Zooplankton Biomass (mg/m^3) vs Chlorophyll-a Concentration (µg/l) in the Water Column

cancelled the registration of chlordane as a pesticide due to its carcinogenicity and persistence in the environment. Also, the National Institute for Occupational Safety and Health has included PCB's and the DDT residues in a list of suspected carcinogens for which further information should be gathered (NIOSH, 1975).

By far, the largest route for the transport of CHC's from the suspended sediment reservoir probably is a result of sediment deposition. The areas of deposition of fine-grain sediments can be visualized as *sinks* where the CHC's attached to suspended sediments collect. As in the Chester River Study (Clarke, et al., 1972), the chlorinated hydrocarbons in the bottom sediments were found highest where the median grain-size diameters were the lowest (Chapter 4, Marine Sedimentology). Baltimore harbor is a *trap* for fine-grain sediments in the upper Chesapeake Bay, and as such, functions as a sink for CHC's from the suspended sediment reservoir. Figure 6-2 shows that the PCB, for instance, was much higher in the sediments of Baltimore harbor than elsewhere in the bay. As will be discussed later in this section, the harbor sediments are probably high in CHC's for more reasons than just their fine grain size. It is necessary to analyze core slices to establish the three-dimentional concentration gradient in order to estimate the mass of CHC's contained in the bottom sediment sinks. (A core from the Chester River was found to contain layers of widely varying levels of CHC's to a depth of 50 centimeters—Clarke et al., 1972.)

A number of mechanisms exist for movement of CHC's out of the bottom sediment sinks. When one considers the fine-grain fractions in the upper Chesapeake Bay as a whole, resuspension of this material by tidal scour probably is the major re-entry route from the sinks to the suspended sediment reservoir (Schubel, 1972). In the Baltimore harbor area, however, maintenance dredging of navigational channels is a major mechanism for removal of sediments containing chlorinated hydrocarbons from the bottom sediment sink. Although the subsequent fate of this dredged material is a subject clouded by great controversy, during the dredging and subsequent overboard discharge of this material, undoubtedly, a substantial quantity returns to the suspended sediment reservoir.

A possible route for CHC movement from the sediment sinks into the biological system could be through deposit-feeding infaunal organisms inhabiting the fine-grain sediments. In particular, certain polychaetes are the most abundant macrofauna in silt-clay habitats including grossly polluted areas in Baltimore harbor (Hamilton, 1972). These organisms form a major part of the diet of crabs and certain species of fish in some areas. The magnitude of this pathway would depend upon the availability of the sediment adsorbed CHC's to the deposit-feeding organisms. (In other words, how much of the adsorbed CHC is stripped off the sediment upon passage through the gut of the organism?) Further, the turnover rates and population densities for the various organisms involved also are important to the magnitude of the pathway. This route is of particular interest because of the direct access to human beings via the crabs and bottom-feeding fish landed for market.

In the investigation of possible sources of chlorinated hydrocarbons, high concentrations were found most often in the samples from two principal areas: (1) Baltimore harbor and (2) Station 1A where the Susquehanna River joins the Chesapeake Bay.

Nearly all of the suspended sediment in the upper bay originates from the Susquehanna River. In a typical year, a major portion of the yearly suspended sediment burden enters the bay during the spring freshet, a period of overpoweringly high flow usually occurring in March or April (Schubel, 1972). The period November 1973 to November 1974 was somewhat atypical in that the spring freshet was not as singular an event as usual. Using the data depicted in Figures 4-32 and 4-33 in Chapter 4, one finds that, during this 12-month period, about 55 percent of the total suspended sediment influx was evenly divided between the two months December and April, with the January, February, March and May influxes totaling another 33 percent (10, 5, 12, and 6 percent respectively). Samples were taken during this program in each of these months except February and April.

Suspended sediments collected at Station 2B April 3 and 4 were analyzed for CHC's under a program for the U. S. Office of Water Resources and Technology. The results were average PCB, 0.31 ppm; average technical chlordane, 0.018 ppm; and average DDTR, 0.027 ppm. (The average ppt values are presented in Chapter 5, Section 5.4.4. A comparison of the concentrations of suspended sediment in the water at the times of sampling (December: 43 mg/l; January: 35 mg/l; March: 11 mg/l; April: 35 mg/l) with the data in Figures 4-30 and 4-31 of Chapter 4 indicate that most of the samples fell near the peak influx periods. The CHC values measured at Station 1A and 2B at high river flow should represent a measure of the degree of contamination of most of the yearly influx of suspended sediments to the suspended sediment CHC reservoir.

An examination of Figures 6-4a, 6-5a, and 6-6a shows that, except for Station 7A in Baltimore harbor, the suspended sediments passing Station 1A during the high-flow conditions appeared to carry sufficient burden of PCB's and DDTR to account for the levels observed at the lower stations without postulating additional sources. The chlordane average appears somewhat low, however, suggesting major inputs other than from the Susquehanna River at high flow periods. Unfortunately, no CHC data were obtained upstream of Station 1A during high-flow conditions, but it seems most probably that CHC contamination on the suspended sediments derives from the watershed of the Susquehanna River rather than from sources at the head of the Chesapeake Bay. The data in Table 6-2, obtained during June and September, suggest that CHC's in the water column on suspended sediments are sufficiently high at Conowingo Dam to account for the levels measured at Station 1A where the Susquehanna River water enters the bay. This point demands further study, however, as one could imagine localized inputs of CHC's from the urban-industrial complex in the region of Station 1A which were keyed to periods of high river-flow.

In light of the mid-portion of the bay typically being characterized by a *turbidity maximum** except for episodes during the infrequent periods of high flow in the Susquehanna River, one would not expect the average CHC concentrations in the water on suspended sediment at Station 1A to be higher than at Stations 5A and 8B, as was found. This atypical situation arises for two reasons: first, the study period was atypical (as discussed above) in terms of river-flow, and second, nearly all of the suspended sediment data presented in Figures 6-4, 6-5, and 6-6 came from samples collected during December, January, March, and May—periods of relatively high river-flow, hence, high suspended sediment load at Station 1A.

Although Baltimore harbor appears to be an estuary of the Patapsco River, the average daily flow of fresh water into the harbor is trivial—about one three-hundredth of the harbor volume (Pritchard, 1968). The harbor has a three-layered circulation pattern. Bay water flows into the harbor in the top and bottom layers, and harbor water flows out in the middle layer, all being driven by the difference between the vertical salinity profile of Baltimore harbor and the Chesapeake Bay (Pritchard, 1968). The rate of this inflow and discharge from the harbor as a result of the circulation pattern was shown to be a fairly constant 17,000 cubic feet per second—about ten percent of the harbor volume per day. (By way of comparison, the 1974 average Susquehanna River stream-flow was 39,900 cubic feet per second.) One could view the harbor as a constant-volume reservoir which is diluted each day by ten percent of its volume with fresh input from the bay, and being constant volume, overflows ten percent of its volume back to the bay.

It has been shown above that the sediments entering the bay from the Susquehanna River carry a chlorinated hydrocarbon burden similar to that found at the other stations except Baltimore harbor. These sediments make up the major part of the suspended sediments in the CHC-suspended sediment reservoir discussed earlier. The water moving into the harbor carries these contaminated sediments; therefore, the waters of the Chesapeake Bay represent a source of the chlorinated hydrocarbons to Baltimore harbor, as was demonstrated for the Chester River (Clarke, et al., 1972). However, several items of evidence suggest that this route may not be the most important source of CHC's to Baltimore harbor.

The fine-grain bottom sediments in Baltimore harbor and the Chester River differ greatly in CHC content—the highest values observed in the Chester River falling well below those found in Baltimore harbor. On this basis alone, one might suppose the existence of local sources in the harbor. However, a sediment core from the Chester River showed two fine-grain layers which had CHC concentrations as high as some of the high values in the harbor sediments. Perhaps the deposition and resuspension processes in the harbor tend to cause an accumulation of fine-grain materials high in CHC's. Even if such a selection process did tend to cause the bottom sediments to approach the high values present in the CHC reservoir (suspended sediment, dry weight, Table 6-3), one still would have difficulty explaining some of the very high Baltimore harbor sediment CHC values without supposing the existence of localized sources, particularly of PCB and chlordane.

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*The turbidity maximum is defined as relatively high suspended sediment concentrations (see Chapter 4, Section 4.2.3.)

An examination of the CHC content of the CHC-suspended sediment—reservoir in Baltimore harbor (Station 7A in Figures 6-4b, 6-5b, and 6-6b) indicate that the amounts of PCB and DDTR present in the water column on suspended sediment are nearly the same as at the other stations outside the harbor. The net flux of these CHC's past the harbor entrance would seem to be zero; hence, at least as much PCB and DDTR would move from the harbor to the bay as is transported from the bay into the harbor. The amount of chlordane adsorbed on suspended sediment in the harbor water column appears to be sufficient to represent a significant source compared to the Susquehanna River, especially when one considers that the flow out of the harbor is almost half as great as the main stream-flow of the river. One cannot be certain from the data whether much of the chlordane actually is transported to the bay with the water returning from the harbor, however. The bottom sediment data certainly indicates that a large portion must be entering the sediment sink in the harbor.

Whether or not Baltimore harbor serves as an important source of chlorinated hydrocarbons to the bay cannot be determined unequivocally from the data gathered during this program. The presence of relatively high levels of petroleum hydrocarbons in the harbor water may solubilize enough of the CHC's to invalidate the assumption that the bulk of the CHC's move adsorbed to the suspended particulates. To resolve this uncertainty one must measure the CHC's in the waters of the harbor as well as in the suspended particulates in the water.

Far from clarifying the pathways of CHC movement in Baltimore harbor, the data presented here and in the other sections serve to indicate the complexity of the situation. In addition to the input from the bay, other inputs of unknown magnitude have been identified (e.g., aerial fallout, rainfall, dust, storm sewer flow). No doubt, additional inputs also would be identified if a pipe-by-pipe point source survey were made. Taken altogether, the data suggest that much of the CHC may be trapped out of the system by the high deposition rate in the harbor. Helz (1974) pointed out that, although the input of trace metals to Baltimore harbor is quite high, these materials appear to be transported only a short distance before being trapped in the bottom sediments.

The few instances when residues of the chlorinated pesticide, toxaphene, were observed during this survey provide interesting support to the supposition that a major portion of the localized inputs of CHC's remained trapped in the harbor. In Chapter 5 (Hydrology and Meteorology), evidence was presented showing that rain water and storm water entering Baltimore harbor are sources of PCB, chlordane, and DDTR. In addition, toxaphene appears to be entering the harbor by these routes. At times, the toxaphene input is sufficient to enter the foodchain—the zooplankton sampled July 8 showed 1.7 ppm toxaphene based upon sample wet weight (0.013 ppt toxaphene in the water column on zooplankton). Apparently this material remains trapped in the harbor, because none of the suspended sediment or zooplankton samples elsewhere in the Chesapeake Bay showed any traces of toxaphene.

As pointed out in Chapter 2, (Marine Biology), Baltimore harbor has a distinctive zooplankton community not found elsewhere in the bay, characterized by a very high population density four to five times greater than the other stations and dominated by a single organism, *Eurytemora affinis.* These organisms could be promoting the deposition of the CHC's from the suspended sediment-CHC reservoir by ingesting and pelletizing the fine-grain materials to form feces and/or psuedo-feces. In addition, if predation upon these organisms were not heavy, the die-off of mature individuals might result in considerable transport of CHC's into the bottom sediments. Studies should be pursued to assess whether significant transport of CHC's occurs into the biological food chain from the bottom sediment sink or the zooplankton standing crop.

The overall CHC movement and distribution in the upper Chesapeake Bay having been discussed, a closer examination of the individual CHC levels found is appropriate. The highest levels of total PCB in zooplankton occurred at the head of the Chesapeake Bay with values of 7.5 and 5.6 ppm at Station 1A in December and March, respectively. The Station 3B zooplankton sample was 7.2 ppm in March. Residues of such magnitude at this trophic level make one wonder what might be found in organisms higher in the food chain.

Two additional zooplankton samples collected at Station 1A were also rather remarkable. The DDTR levels found in zooplankton were 4.2 ppm in June and 3.9 ppm in September. These astoundingly high levels (compared to elsewhere in the bay) were 97 percent DDD (most p,p'-DDD with some o,p'-DDD). This mixture could not result from the breakdown of DDT, but insead it most likely represents the use of DDD itself. (For instance, a mixture of the p,p' and o,p' isomers of DDD has been marketed as a pesticide under the trade name *Rothane*®.) These occurrences do not appear to indicate a major source of

DDD to the bay; while DDD is the most abundant DDT residue found in the upper Chesapeake Bay, this occurrence is not unusual for an estuarine environment. The absence of particularly high DDD levels in the Conowingo Dam samples taken in June and September might assure one that the material did not come from upstream of the Dam, except that high DDD was not present in the suspended sediment samples at Station 1A at these times either. A single event of this nature might be dismissed as interesting but probably not significant. However, the magnitude of the values and their repeated occurrence seem to indicate a situation requiring further study.

Aside from the very high CHC levels found in the bottom sediments in Baltimore harbor, the bottom sediments in the mouth of the Gunpowder River also were very high in PCB (1.3 ppm) and DDTR (0.27 ppm) compared to elsewhere in the upper bay. Access to this river is limited because it is part of the U. S. Army's Edgewood Arsenal/Aberdeen Proving Grounds complex. Therefore, only the sediments at the river mouth were sampled and analyzed. Although, further sampling would be necessary to determine whether this area provides major amounts of CHC's to the bay, the values at the mouth of the Gunpowder River are too high to be a result of the suspended sediment deposition from the bay. Rather, they indicate localized sources of PCB and DDTR.

6.5 Conclusions

1. Relatively high levels of the chlorinated hydrocarbons, PCB, chlordane, and DDTR were found in the samples from the upper Chesapeake Bay. CHC's in bottom sediments from the bay were two to three times higher than those found in the Chester River.
2. The bottom sediments from Baltimore harbor were found to contain the greatest amount of the CHC contaminants with values as high as 3.7 ppm for PCB, 0.082 ppm for chlordane, and 0.19 for DDTR.
3. The average concentrations of PCB, chlordane, and DDTR were highest in suspended sediments filtered from the water in Baltimore harbor, with high individual values of 3.8 ppm for PCB, 0.34 ppm for chlordane, and 0.30 ppm for DDTR being found. When these data are presented as the amount of CHC present in the water column on suspended sediment, the average CHC values show a decreasing trend going down the Chesapeake Bay with the Baltimore harbor station not as high in the case of PCB, or about the same as the upper stations in the case of DDTR and chlordane. The chlordane is higher in the harbor than elsewhere in the bay.
4. The CHC residue concentrations found in zooplankton samples varied tremendously in time and space—so much, that the sampling frequency probably was not sufficiently high, temporally or spatially, for the average values to be entirely representative. The concentrations of PCB and DDTR in the zooplankton were highest at Station 1A at the head of the Chesapeake Bay (high values of 7.5 ppm PCB and 4.2 ppm DDTR). The most consistently high chlordane values in zooplankton were found in Baltimore harbor, the highest being 0.14 ppm.
5. A positive correlation was found between the concentration of suspended sediments in the water and the concentration of CHC's in the water column on suspended sediments.
6. A positive correlation was also found between the zooplankton biomass in the water and the CHC concentration in the water column on zooplankton.
7. Apparently, the CHC's enter the Chesapeake Bay attached to suspended sediments via the Susquehanna River during periods of high river-flow. These suspended sediments appear to function as a reservoir for CHC's in the upper bay.
8. The principal movement from this reservoir probably is into bottom sediments which could be considered sinks or traps for sediment-adsorbed chlorinated hydrocarbons.
9. Resuspension probably represents the greatest pathway for movement of sediment adsorbed CHC's from the sinks, although re-working of sediments by deposit-feeding organisms is a possible route which needs further study.

10. The amount of chlorinated hydrocarbons passing into the zooplankton community appears limited only by the zooplankton population density rather than by fluctuations in the reservoir, because the amount of CHC present in the zooplankton standing crop never represents more than a small fraction of that present in the suspended sediment reservoir.
11. Another major pathway from the reservoir into the biological system is into the shellfish populations of the upper Chesapeake Bay.
12. Baltimore harbor receives CHC's from the surrounding urban-industrial complex and from exchanging water and suspended sediments with the bay. Although the harbor appears to be a significant source of chlordane, it is not clear whether a net transport of PCB and DDTR from the harbor into the bay occurs via the continuous exchange of harbor and bay water.
13. The toxaphene observed in the rainfall and storm sewer samples appears to enter the food chain only in Baltimore harbor, and there only sporadically.
14. The data showed bio-concentration of the CHC's by the plankton and shellfish in the bay. The zooplankton concentrated the CHC's five to eight times over that found in the suspended particulate fraction. The shellfish concentrated the CHC's many thousands of times higher than the levels in the water column (PCB, 4,000; chlordane, 30,000; DDTR, 45,000).

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