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Monitoring Guidance for the National Estuary Program

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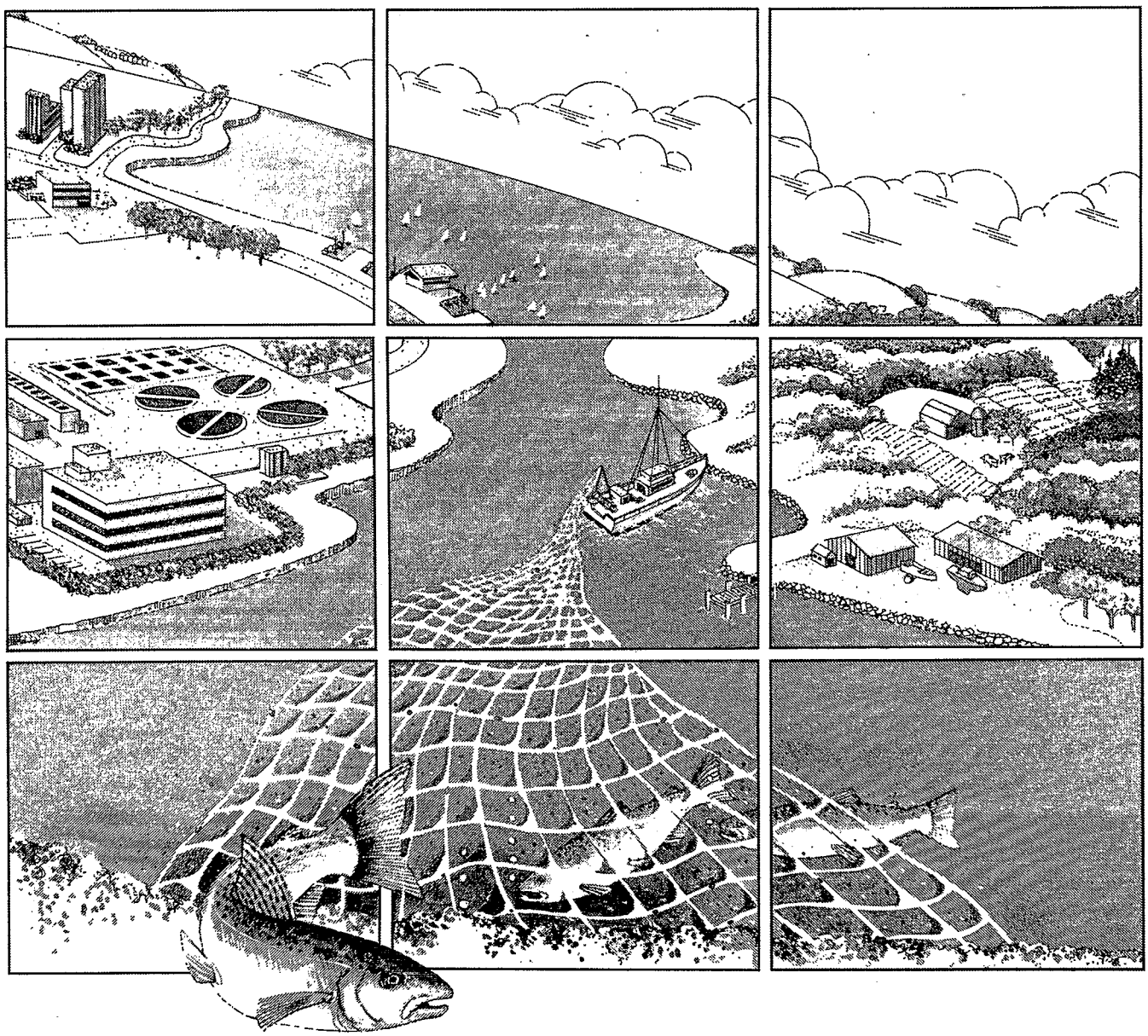

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MONITORING GUIDANCE FOR THE NATIONAL ESTUARY PROGRAM

Final

Office of Water
Office of Wetlands, Oceans, and Watersheds
Oceans and Coastal Protection Division
U.S. Environmental Protection Agency
Washington, D.C. 20460

September 1992

Executive Summary

Purpose

This document provides the National Estuary Program (NEP) with guidance on how to design, implement and evaluate a monitoring program. The careful use of this document will lead to more complete information to measure the success of management actions initiated under the Comprehensive Conservation and Management Plan (CCMP) as well as essential data to evaluate environmental risks and trends. It could also be useful to other coastal and marine managers with monitoring responsibilities.

The National Estuary Program

In 1987 Congress amended the Clean Water Act and created the NEP to recognize nationally significant estuaries, to protect and improve their water quality, and to enhance their living resources. The Environmental Protection Agency (EPA) has selected 18 estuaries under the NEP and has plans to add more.

One of the major products of each estuary program is the CCMP which identifies priority problems, determines environmental quality goals and objectives, and specifies action plans and compliance schedules to ensure that designated uses of the estuary are protected. Included in the CCMP is a NEP monitoring plan.

National Importance

A recent report by the National Research Council (*Managing Troubled Waters, The Role of Marine Environmental Monitoring;* NRC, 1990) recognized the need for vast improvements in marine monitoring. Separately, the Expert Panel on the Role of Science at EPA established by the EPA Administrator in 1991 challenged the EPA to acquire and to use the best scientific information in the decision making process of EPA program offices (*Safeguarding the Future: Credible Science, Credible Decisions; The Report of the Expert Panel on the Role of Science at EPA*). This guidance document incorporates the recommendations of both national reports. Furthermore, the EPA Science Advisory Board commended this guidance for its systems approach to monitoring and the use of objective-driven criteria in designing monitoring programs.

Highlights

A monitoring program should meet three interrelated objectives: verify the predicted responses of the estuary to management actions, measure the effectiveness of programs implemented under the CCMP, and provide essential information that can be used to redirect and refocus the CCMP.

To meet these objectives, five steps are outlined in the monitoring design framework:

Step 1 Develop Monitoring Objectives and Performance Criteria

Explicit statements of the monitoring objectives and performance criteria with which to measure monitoring program success are crucial. Example monitoring objectives, performance criteria, and tools for developing the objectives are presented.

Step 2 Establish Testable Hypotheses and Select Statistical Methods

Establishing testable hypotheses ensures that the results of the monitoring program will be unambiguous and that the objectives of the program can be met. To guarantee sufficient information and the right type of information, the NEPs should choose the statistical model that will be used to analyze the field data and testable hypotheses prior to the collection of any samples. References on the use of various statistical methods are provided.

Step 3 Select Analytical Methods and Alternative Sampling Designs

Detailed specifications for each monitoring variable of the monitoring program must be developed. These include field sampling methods, laboratory procedures, and QA/QC procedures. Alternative sampling methods are described, and the use of data from existing monitoring programs is discussed.

Step 4 Evaluate Expected Monitoring Study Performance

During the course of the monitoring program design and execution, performance evaluations provide a systematic procedure for measuring success in terms of the ability to meet program goals. Statistical tools for evaluating program performance are described, and the use of feedback mechanisms are recommended to refine objectives and to improve monitoring program performance.

Step 5 Implement Monitoring Study and Data Analysis

Data management and data analysis, key issues that are often overlooked in the design of monitoring programs, are as important to the success of the monitoring effort as the collection and laboratory analysis of field data. Data and information management issues are addressed, and the importance of the analysis and the communication of program results are discussed.

Two case studies from existing estuarine monitoring programs (i.e., Puget Sound Ambient Monitoring Program and Chesapeake Bay Monitoring Program) are presented which demonstrate the process of developing a strategy and the use of statistical methods to evaluate the monitoring plan before and after implementation. Also, sampling and analytical methods available for monitoring estuarine water quality, sediment quality, biological resources and human health risks are presented.

NEP's environmental monitoring activities depend on integrating state, federal and local activities. The NEP is encouraged to coordinate with existing monitoring efforts such as the EPA's Ecosystem Monitoring and Assessment Program (EMAP), the National Oceanic and Atmospheric Administration's National Status and Trends Program, and the U.S. Geological Survey's National Water Quality Assessment Program.

A companion to this document (*Technical Characterization Guidance for the National Estuary Program*) will be released soon. The characterization guidance will help NEPs identify and set priorities among problems within the estuary during CCMP development.

Acknowledgments

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1.0 Introduction

The Clean Water Act as amended by the Water Quality Act of 1987 establishes the National Estuary Program (NEP) to promote long term planning and management in nationally significant estuaries threatened by pollution, development, or overuse. Section 320 of the Clean Water Act describes the establishment of a management conference in each estuary to develop a Comprehensive Conservation and Management Plan. It also establishes requirements to monitor the effectiveness of actions taken pursuant to the plan.

This document provides guidance on the design, implementation, and evaluation of the required monitoring programs. This document should also be of use to others developing monitoring programs. The intended audience is the members of the management conference (i.e., the Management Committee, Scientific and Technical Advisory Committee, and the Citizens Advisory Committee) and the program coordinators and scientific staff of the individual estuary programs. The purpose of this document is to identify the steps involved in developing and implementing estuarine monitoring programs. Given the large number of participants in the management conference and the diversity of technical backgrounds, it is also intended to provide a technical basis for discussions on the development of monitoring program objectives, the selection of monitoring program components, and the allocation of sampling effort. Some of the issues addressed in this document are:

- What is the role of monitoring in the estuary programs?
- Why are monitoring programs necessary, and what are examples of goals and objectives?
- What criteria should be used to select components of the monitoring program, and what should drive the decision-making process?
- What is the importance of historical data, and how can ongoing monitoring programs be incorporated into the estuarine monitoring program?
- What is the relationship between estuary characterization and the monitoring program?

The key technical and programmatic aspects associated with each of these issues are described. This document also includes several examples. Two case studies are used to provide examples from existing estuarine monitoring programs. While these case studies focus on unique sets of problems and questions from individual programs, they

provide illustrations of the process of monitoring program design, implementation, and evaluation. The lessons learned are of general applicability even though the political and institutional setting may be different from other estuaries.

The first case study from the Puget Sound Estuary Program (Appendix A1.0) provides an example of the process of developing an effective monitoring strategy. Key issues addressed include problem and goal definition, the process of monitoring program design and implementation, and options for funding estuary monitoring programs. The first case study also demonstrates how ongoing monitoring studies can be coordinated to develop a comprehensive basin-wide monitoring program. The second case study (Appendix A2.0) provides a detailed example of the application of methods for determining the effectiveness and feasibility of monitoring efforts in the Chesapeake Bay Program. Examples from other environmental monitoring programs are also described.

Appendix B of this document describes existing methods that may be used to monitor the effectiveness of management actions in the estuary. The National Estuary Program does not impose national requirements for standard methods. However, emphasis is placed on the importance of using standardized monitoring protocols within each estuary and developing performance-based criteria to evaluate the comparability of analytical methods. One long-term goal of the NEP is to develop a standardized set of key variables that, if measured in all NEP programs, will provide comparative data on nationally significant estuaries.

Funding is typically drawn from federal, state, and local sources for National Estuary Program monitoring activities. Therefore, this document discusses the integration of existing monitoring efforts into the estuary monitoring program. It is also essential that National Estuary Program monitoring activities be coordinated with existing federal agency status and trends monitoring programs such as EPA's Ecosystem Monitoring and Assessment Program (EMAP), the National Oceanic and Atmospheric Administration's (NOAA) Status and Trends Program, and the U.S. Geological Survey's National Water Quality Assessment Program. If individual estuary monitoring programs will be measuring the same parameters as these federal programs, and the protocols in use provide adequate data, estuary programs should work to ensure compatibility of their data with the existing federal programs.

Data management, effective data analysis, and the communication of monitoring program results to a wide range of audiences at several technical levels are also essential to the success of the estuary program. This document discusses data manage-

ment strategies and provides several examples of ongoing efforts to disseminate information developed by the National Estuary Program.

1.1 Background

National Estuary Program

The purpose of the NEP is to identify estuaries of national significance and to promote the preparation of comprehensive management plans to ensure their ecological integrity. The process of identifying nationally significant estuaries and nominating estuaries to become part of the NEP is described in *A Primer for Establishing and Managing Estuary Projects* (U.S. EPA, 1989a) and *Final Guidance on the Contents of a Governor's Nomination* (U.S. EPA, 1990a). Following selection of an estuary to be included in the National Estuary Program, a management conference is convened by the EPA Administrator. The management conference has the responsibility to implement a four-phased program:

- Phase I – The Planning Initiative. The planning phase is intended to build the management framework for identifying and solving problems and defining the steps in the decision making process.

- Phase II – Characterization and Problem Definition. The goal of estuary characterization is to gather and summarize the existing knowledge concerning the state of the estuary as well as the physical, chemical, and biological factors controlling spatial and temporal changes. The characterization process focuses on identifying existing and potential problems and exploring probable causes of such problems. Such cause and effect linkages between human activities and environmental changes provide the public and decision makers with the information necessary to develop priorities, set management strategies, and devise mitigating measures. However, information gaps do exist. Such information gaps can be filled by additional sampling and a subsequent monitoring program. The characterization process is described in a separate EPA guidance document.

- Phase III – Development of a Comprehensive Conservation and Management Plan (CCMP). The CCMP is a major product of the estuary program. It is developed by the management conference to summarize findings, identify and prioritize problems, determine environmental quality goals and objectives, identify action plans and compliance schedules for pollution control and resource management, and to ensure that designated uses of the estuary are protected.

- Phase IV – CCMP Implementation. When the final CCMP is submitted for approval to the administrator, the management conference and the governor establish a committee to coordinate the implementation of the CCMP. The development of a monitoring program to evaluate the effectiveness of actions specified in the CCMP is a required task of the management conference.

The four phases of the NEP should *not* be viewed as sequential steps, where one phase cannot be initiated before another phase is completed. On the contrary, as the NEP has evolved, EPA has encouraged management conferences to proceed with the four phases simultaneously as often as possible. For example, early results of characterization (Phase II) may indicate obvious management actions that can be taken (Phase IV) prior to completion of the CCMP (Phase III), In these cases, implementation actions should proceed. In fact, EPA will base the selection of any new estuaries on an ability to streamline the NEP phases, focusing on estuaries that have a significant amount of the problem characterization done, that have a successful management framework that functions similarly to an NEP management conference already established, and that have clear commitments from key state and local agencies to participate in and support the NEP process.

Environmental sampling is required in Phases II and IV of the estuary programs. The studies conducted in Phase II are focused on filling identified data gaps, providing baseline data on point and nonpoint loadings of pollutants, and developing estimates of the degree of spatial and temporal variability. Sampling is conducted in Phase IV of the estuary programs as part of a long term environmental monitoring strategy.

Environmental *monitoring* that is conducted during Phase IV is considered to be different from the *sampling* that is conducted during Phase II. Monitoring involves repeated sampling over time. For example, short term sampling may be conducted in Phase II to collect specific information on the concentration, distribution, and variability of chemical contaminants in sediments. The goal of the corresponding sampling that is conducted during Phase IV is to evaluate trends in monitored variables and to link the observed patterns to specific management actions. A second distinction between Phase II sampling and Phase IV monitoring is that comprehensive environmental monitoring programs conducted during Phase IV will require the integration of information from several concurrent sampling efforts. Since environmental sampling is costly and resources will be limited, there will be a need to evaluate the efficacy of different monitoring program components and to allocate sampling efforts accordingly. It will also be necessary to carefully plan and coordinate monitoring efforts among individual monitoring components and other preexisting monitoring programs

to determine interactions and streamline monitoring efforts. A third distinction between environmental sampling and monitoring is the need to periodically analyze the monitoring program results and modify the level of sampling effort to maximize program effectiveness.

NEP Monitoring Program Goals

EPA is developing a programmatic monitoring system for NEP participants to use in tracking the progress being made in protecting NEP estuaries. While primarily aimed at tracking implementation of actions recommended by the management conferences in their CCMPs, the system will be helpful in the assessment of the NEP in its entirety. This programmatic monitoring system will:

- Assist estuary program managers to improve their programs by identifying current and emerging programs;
- Provide accountability to elected officials and the public relating to the progress toward estuary protection;
- Help identify the programs and projects that are working well; and
- Provide a framework for assessing the NEP as a whole.

This programmatic monitoring system will identify estuary management performance indicators that will track, on a regular basis, the status and progress of action-oriented efforts. Much of the input to this programmatic monitoring system will come from information generated by NEP environmental monitoring programs implemented under Phase IV of the NEP, as well as other environmental monitoring programs.

The two primary goals of the monitoring programs implemented in Phase IV by the management conferences are:

- To measure the effectiveness of management actions and programs implemented under the CCMP and
- To provide essential information that can be used to redirect and refocus the management plan.

These monitoring programs are an essential part of the management program review and evaluation process for each estuary, and they will be conducted throughout the implementation of the CCMP.

This document describes the steps involved in designing an environmental monitoring program to meet these two interrelated goals. For example, the goals of the Puget Sound Ambient Monitoring Program (described in Case Study 1) include the characterization and interpretation of spatial and temporal patterns of conditions in Puget Sound, development of a permanent record of significant natural and human-caused changes in key environmental indicators, and the support of research activities through the availability of consistent, scientifically valid data. Such research will lead to a better understanding of the state of the estuary in question and, in turn, provide information for making better resource management decisions.

Monitoring Design Issues

> **Marine monitoring is the continued, systematic time-series observation of predetermined pollutants or pertinent components of the ecosystem over a period of time sufficient to determine (1) the existing level, (2) trends, and (3) natural variations of measured components (NOAA, 1979).**

Recent reports by the National Research Council (NRC, 1990a and 1990b) evaluated current marine monitoring programs and practices, identified needed improvements in monitoring strategies, and made a series of recommendations to improve the usefulness of monitoring information.

The extensive NRC review of monitoring practices found numerous inadequacies in the design and use of monitoring data. Three broad problem areas were identified:

- Monitoring programs are often poorly designed because of failure to clearly define monitoring objectives and to apply available design tools. Some of the identified problems could be attributed to the inherent difficulty of separating the effects of human activities from natural variability.

- There is a lack of communication and coordination among the regulatory, scientific, and management entities sponsoring or conducting monitoring programs. Specific concerns include the inflexibility of regulatory requirements that limit opportunities to adapt programs to new needs and regional

objectives. The need to adopt standardized sampling and quality assurance procedures to ensure data comparability has also been identified.

- The results of monitoring programs are not presented in a form that is useful in developing broad public policy or evaluating specific control strategies. It is essential to link data management strategies and data analysis methods to the objectives of the monitoring effort. It is also necessary to devise a plan for effectively communicating monitoring results to the identified audience.

Additionally, the NRC study found that poorly designed monitoring programs and the lack of communication and coordination among programs has often resulted in:

- Limitation of water quality and public health monitoring to areas adjacent to point-source outfalls and other known or potential problem areas, and to the sampling and analysis of conventional water quality and public health parameters

- Living resource monitoring programs restricted to assessment of a few higher-trophic levels of commercially or recreationally important species, without consideration of key prey species upon which these species depend

- Incomplete monitoring of living resources and habitats key to the determination of potential declines in living resources and water quality (i.e., near shore habitats, estuarine wetlands, plankton communities)

1.2 Recommended Monitoring Design Procedures

A wide range of guidance on the design of environmental monitoring programs is available in the literature. Important contributions have been made that address the principles and options for designing monitoring networks (Green, 1979; Segar and Stamman, 1986), the development of monitoring objectives (Beanlands and Duinker, 1983), the appropriateness of monitoring variables (Bilyard, 1987), and the application of statistical methods in the design process (Ferraro *et al.*, 1989). The recent NRC study (NRC, 1990a) also provides comprehensive review of the steps involved in the design of marine environmental monitoring programs.

Outlined below is a systems approach to the design of the required NEP monitoring programs that incorporates existing information and that will ensure the collection, analysis and reporting of adequate information to meet the goals of individual estuary programs.

TABLE 1-1. ELEMENTS OF SYSTEMS APPROACH

• Define the Objective	– The overall objective of the design process is stated in a succinct manner.
• Establish Information Needs	– Information requirements to meet the objectives are established.
• Establish the Objectives of Individual Program Components	– The objectives of all possible monitoring program components and performance criteria are established.
• Evaluation of Trade-Offs	– The combination of monitoring components that best meet the overall objectives is selected.
• Feedback to Initial Design Step	– Modifications to the system's design are made to improve the product's performance.

A systems design approach places emphasis on the optimum design of the overall monitoring program. The essential elements (shown in Table 1-1) are the assessment of trade-offs between individual aspects of the monitoring program and the use of feedback mechanisms to modify individual monitoring program components based on periodic assessments of overall program performance. This approach is well suited for design problems that involve complex, highly variable systems, such as estuaries, and that involve a large number of investigators that must interact as a group to produce the product.

The plan for implementing this approach to designing the monitoring program is shown in Figure 1-1. It involves the specification of monitoring objectives, the completion of a series of steps to translate these objectives into clearly defined monitoring activities, and the use of feedback mechanisms to refine the objectives and adjust the sampling effort.

The overall objective of monitoring undertaken during Phase IV of the estuary programs will be to measure the effectiveness of management actions implemented as part of the CCMP. Meeting this broad program objective will require the specification of several individual, highly interrelated *monitoring objectives.* Examples of individual monitoring objectives (shown in Figure 1-1 as Objectives 1 through n) include: to determine the response of key water quality variables to management actions; to

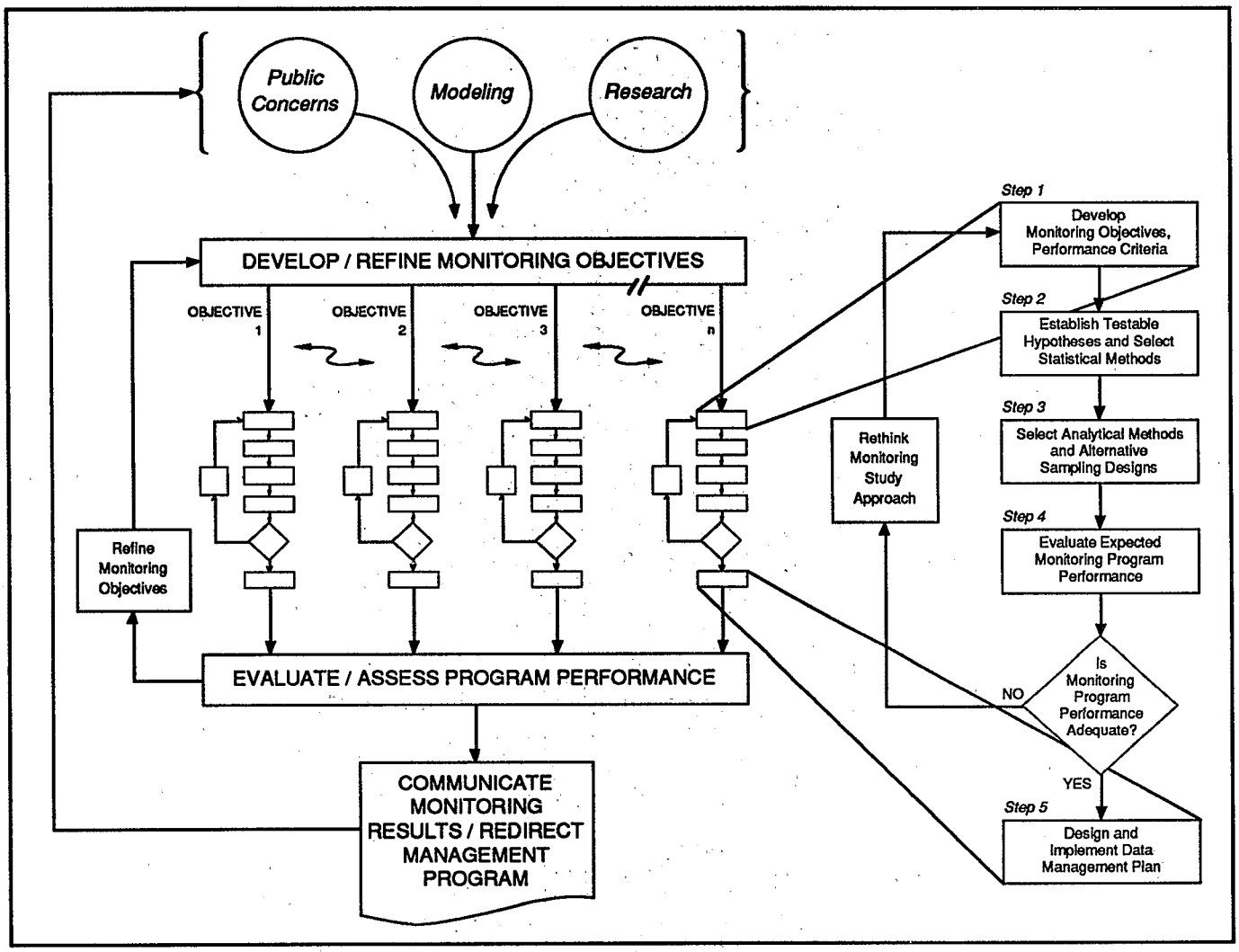


Figure 1-1. Monitoring program design.

determine trends in sediment contaminant concentrations; and, to evaluate the persistence of PCBs in the tissue of recreational and commercial fish.

Each monitoring objective represents a separate component of the overall estuary monitoring program. The individual steps involved in designing each component of the monitoring program are shown on the right-hand side of Figure 1-1:

- *Step 1.* Develop Monitoring Objectives and Performance Criteria. Clear objectives and corresponding performance criteria must be developed for each component of the monitoring program. Performance criteria specify the level of change or trend that the monitoring program must be able to

detect. For example, in the Chesapeake Bay Program, a target of 40% reduction in nutrient loads to the bay was established based on modeling results. One of the predicted benefits of nutrient reduction is the alleviation of anoxic conditions in the bay and an increase in the minimum dissolved oxygen to approximately 1.5 mg/ℓ. Therefore, the corresponding objective and performance criterion for the water quality component of the monitoring program would be to detect a long term change in dissolved oxygen concentration in the bottom waters of the bay equal to 1.5 mg/ℓ.

- *Step 2.* Establish Testable Hypotheses and Select Statistical Methods. The study objectives must be translated into statistically testable hypotheses; for example, no trend exists in measured values of dissolved oxygen concentration. Establishing testable hypotheses ensures that the results of the monitoring program will be unambiguous and that the objectives of the program can be met. The establishment of testable hypotheses also guides the development of statistical strategies for determining sample locations and times as well as the selection of statistical tests that will be used to analyze the resulting data.

- *Step 3.* Select Analytical Methods and Alternative Sampling Designs. Detailed specifications for each monitoring variable (measurable endpoint) of the monitoring program must be developed. These include: field sampling and laboratory procedures, and QA/QC procedures. Additionally, alternative sampling designs that specify the number and location of stations must be devised for input to the next step in the design process.

- *Step 4.* Evaluate Expected Monitoring Program Performance. It is essential to evaluate the expected performance of the initial sampling design to determine the minimum difference that can be detected over time or between locations. Without this evaluation there is a risk of collecting and analyzing too few samples to detect statistically significant temporal or spatial trends or of analyzing an excessive number of samples (with associated high costs). As indicated by the feedback loop shown in Figure 1-1, the results of this evaluation are used to identify modifications to the initial design in order to increase monitoring program effectiveness. Information from this evaluation will also be used to assess the ability of monitoring components to provide information used to modify the management plan.

- *Step 5.* Design and Implement Data Management Plan. The development of a data management system is an essential task that is often overlooked in the design of monitoring programs. The data management system should be operational prior to implementation of the monitoring program. Data analysis methods and a timetable for analyzing the data, assessing CCMP implementation progress and monitoring program performance, and reporting program results should also be specified. The results of the performance assessment are used to refine program objectives and modify individual study elements to satisfy these objectives.

These individual steps in the monitoring design process shown in Figure 1-1 are described in Sections 2 through 6 of this document.

Peer review of the monitoring program is recommended to evaluate and assess program design. Critical review by technical experts without a vested interest in the estuary program will ensure that the monitoring objectives are meaningful and that the monitoring strategy adequately addresses these objectives with the most appropriate methods. This review should take place after the initial development of specific objectives and performance criteria and as part of periodic reviews that are conducted to evaluate the success of the monitoring program.

Overall program performance must be assessed at periodic intervals, and the results should be used to refine monitoring program objectives and methodologies. The original monitoring design must remain open to modification. The monitoring program should take advantage of new information and innovative sampling approaches as they are developed, and the link between modeling and monitoring efforts should be fully exploited. The results of the monitoring program should be used to refine and modify conceptual and mathematical models of the system, and modeling results should be used to guide changes in individual monitoring program components, variables monitored, the frequency of sampling, and overall monitoring strategies. It is essential that new information, from both independent research and the monitoring program results, is integrated into the monitoring program.

1.3 Monitoring Program Management

There are a number of management issues related to the design, implementation, and maintenance of NEP monitoring programs that must be addressed early by the management conference. These include setting the timetable for the design and implementation process, assigning responsibilities for coordinating the design effort, and planning for the long term success of the monitoring program.

Timetable for the Design and Implementation of the Monitoring Program

The design and implementation of the Puget Sound Ambient Monitoring Program (PSAMP), described in Case Study 1, required four years of uninterrupted effort. The development of the monitoring program was a consensus building effort among numerous agencies and organizations, and several iterations were required to finalize the initial design plans. In addition to the decisions regarding the basic sampling strategy, agreement was required on numerous interrelated issues, such as sampling protocols, appropriate quality assurance/quality control methodology, and the selection of an information management system.

Planning for the monitoring program should be initiated during the first year of the management conference. Milestones for the monitoring program effort should be clearly stated in the State-EPA Management Conference Agreement (a three to five year action plan for CCMP completion that is negotiated shortly after a management conference is convened). Development of the monitoring program should be given a high priority by the management conference after it is convened. It is important to begin early planning of the monitoring program to ensure that it is in place at the time the CCMP is implemented. A detailed monitoring program plan must accompany the CCMP that is submitted to the EPA Administrator for approval. The monitoring plan must contain the following elements that are described in this document:

- Definition of program objectives and performance criteria (parameter values needed to guide management decisions)
- Identification of testable hypotheses
- Detailed specifications for each monitoring variable, including sampling locations and frequency, field sampling procedures, field and laboratory analytical procedures, quality assurance and control procedures
- Specification of the data management system and statistical tests that will be used to analyze the monitoring data
- Description of the expected performance of the initial sampling design (i.e., the minimum difference that can be detected in measured variables over time and between locations)
- Plan and timetable for analyzing data and assessing program performance

Management Tasks for Developing the Monitoring Program

The first management task in developing the monitoring program should be the establishment of an organization or committee, such as a monitoring subcommittee of the Scientific and Technical Advisory Committee (STAC), to develop the monitoring program. The STAC is one of three committees that make up the NEP management conference. The other two committees are the management committee and the Citizen's Advisory Committee. Membership in the monitoring subcommittee should not be limited to STAC members but should include representatives from federal, state and local agencies, universities, industry, environmental groups and others currently conducting monitoring or planning monitoring programs within the estuary or surrounding waters. The subcommittee should be charged with the following tasks:

- Define the goals and objectives of the monitoring program.
- Propose an initial design that includes recommendations for sampling and analytical protocols, data management system specifications, quality assurance guidelines, data reporting requirements and cost estimates.
- Develop the final monitoring design using workshops and other mechanisms to solicit comments and suggestions from the public and scientific community.
- Coordinate the activities of the numerous interested and participating agencies and develop interagency agreements that will promote the monitoring effort.
- Identify funding mechanisms and opportunities to contain costs.

As discussed in Section 1.2, the process of developing a comprehensive monitoring program must begin with a clear statement of the objectives. The explicit statement of objectives, and options for obtaining these objectives, is necessary as a starting point for describing the problem areas in the estuary and developing the consensus among interested agencies and other parties that is essential to the success of the monitoring effort.

The primary goals of the monitoring program will be to measure the success of the CCMP and to provide information that can be used to redirect and refocus the management. However, it will also be the responsibility of this subcommittee to develop secondary goals and program objectives that will be used to focus the sampling effort.

These objectives could include: continued characterization of spatial and temporal patterns of change in water quality, sediment and biological resources of the estuary; development of a permanent record of significant natural and human-caused changes in environmental indicators in the estuary over time, and support for research activities through the availability of consistent, scientifically valid data. The process of developing these objectives is described in Section 2.0.

There are several options for initiating the design process. The PSAMP began with an initial monitoring design developed by consultants to EPA Region 10. The initial design included goals and objectives, plans for operation of the program, and methods for sampling, analysis, and reporting the data. The first draft of the monitoring program design was refined by EPA Region 10 through a process involving meetings and discussions among managers and scientists working in the Puget Sound region. The Puget Sound Monitoring Management Committee then proceeded to review, modify and refine the proposed monitoring design. This resulted in the development of a revised draft that was released for public and further scientific review. The Santa Monica Bay Restoration Project began the development of the monitoring program with an "Assessment of Monitoring and Data Management Needs Workshop" to develop and evaluate monitoring options. In preparation for the workshop, background information was compiled and used to develop a "straw man" to guide workshop discussions. In both the Puget Sound and the Santa Monica programs, public and scientific workshops were held to discuss the proposed monitoring options and to build the required consensus among participating entities.

The next task following the adoption of the monitoring program design is to ensure that it is implemented as planned. The implementation of a regional or basin-wide monitoring program requires the development of commitments and the coordination of activities among the participating agencies. This was achieved in the Puget Sound Ambient Monitoring Program by negotiating memoranda of agreement (MOA) with each of the participating agencies. The MOAs specified commitments that each agency carry out its responsibilities identified in the monitoring program design, and that each agency maintain monitoring program funding levels and staff support for the monitoring program committees. As described in Case Study 1, the negotiating of the MOAs was essential to the success of the PSAMP, but the complexity of the process was underestimated. It is recommended that the coordination efforts begin as soon as possible in the design process.

It is also important that the monitoring subcommittee begin to identify, early in the process of monitoring program development, the costs associated with existing monitoring in the basin, the costs of additional monitoring that will be needed, potential

sources of additional funds to support the monitoring program, and appropriate mechanisms to fund the monitoring program. Costs of the Puget Sound Ambient Monitoring Program were calculated by a technical costing subcommittee of the monitoring management committee with the assistance of a technical consultant.

The estimates provided by this subcommittee demonstrate that the costs of comprehensive monitoring programs can be substantial. In addition to the $200,000 in staff and consultant time required to develop the monitoring program design, the calculated costs of full implementation of the PSAMP are approximately $3.2 million per year (in 1987 dollars). The initial sampling program was reduced in scope due to resource constraints, and costs for the program have been $250,000 to $350,000 over the first two years. The projected costs of implementing the monitoring plan submitted as part of the Buzzards Bay CCMP are $750,000 per year. This includes $200,000 funded by the estuary program to supplement ongoing state and citizen monitoring programs. The costs of the comprehensive water quality monitoring in the mainstem of the Chesapeake Bay are on the order of $900,000 annually. This monitoring includes the sampling of 20 variables at 49 stations, 20 times. In addition to mainstem sampling, the states surrounding the bay have undertaken their own extensive sampling programs in the bay's tributaries.

The substantial costs of these programs require equally substantial efforts to secure long term funding. Most of the costs of monitoring should be borne by the state and local agencies that will complete specific monitoring tasks. Therefore, the best opportunity for securing the required funding is indirectly by incorporating existing federal, state and local monitoring programs into the overall design. The critical step will be to motivate the participating agencies to make modifications to existing monitoring efforts to meet the goals of the estuary program. The best way to achieve this objective is to involve these agencies in the estuary program and the monitoring design process as early as possible and to demonstrate the benefits of a regional / basin-wide monitoring program to the scientific and regulatory community. If state and local funds are not immediately available for full implementation of the monitoring program, a plan for phased implementation can be developed through a priority setting process involving the monitoring management committee and other technical experts.

Section 4.0 of this document discusses the incorporation of individual components of ongoing local monitoring programs into the National Estuary Program monitoring efforts. Opportunities for taking advantage of monitoring programs of national scope, such as EPA's Environmental Monitoring and Assessment Program (EMAP) and NOAA's Status and Trends Program, as well as locally coordinated citizen monitoring programs, are also discussed.

Financing Marine and Estuarine Programs: A Guide to Resources (U.S. EPA, 1988a) provides guidance for obtaining direct financing of marine and estuarine monitoring programs. This document provides a primer on basic financing concepts and explains the initiatives needed to begin financial planning for long term resource management activities. Case studies are also included that provide examples of local financial efforts.

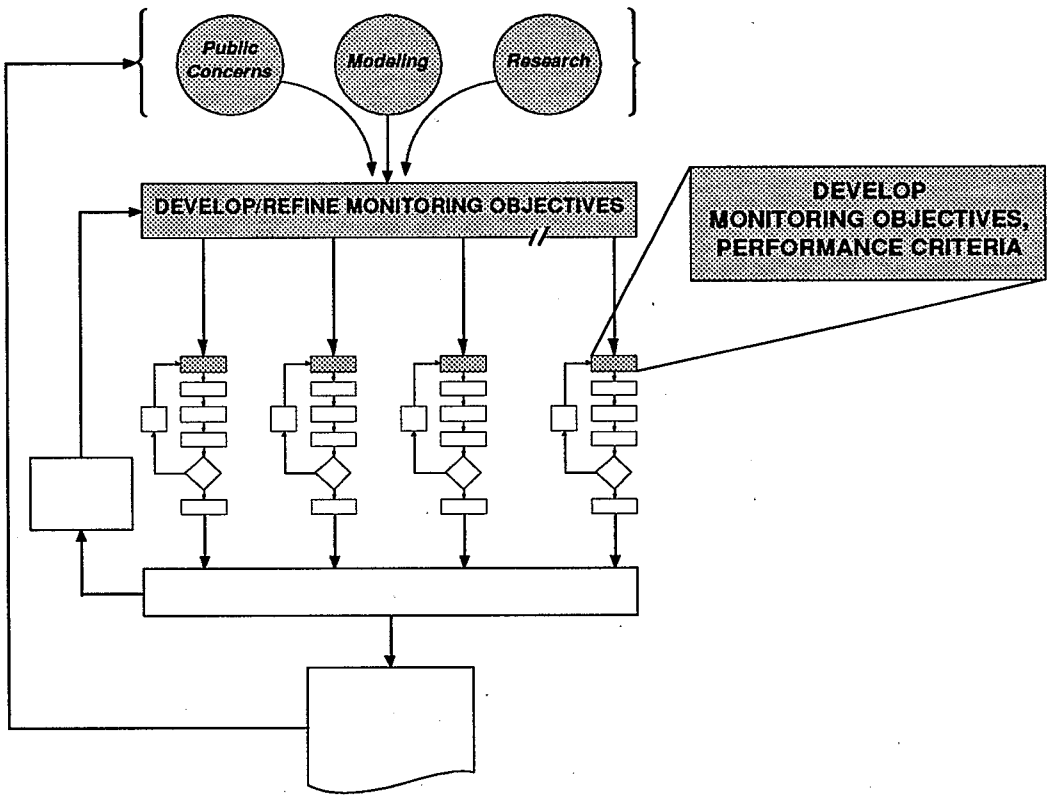
Planning for the Long Term Success of the Monitoring Program

A structure for managing the monitoring program must be recommended to the management conference by the monitoring subcommittee or its equivalent. A lead coordinating agency or organization must be identified and given the responsibility for implementing the monitoring design as planned, maintaining the monitoring effort, and reviewing the monitoring program results. The lead agency or organization should provide a full-time manager/coordinator and a staff that is responsible for keeping all the implementing agencies and other participants informed of progress, resolving disputes, carrying out technical and administrative duties, and reporting the results of the program.

2.0 Develop Monitoring Objectives and Performance Criteria

The overall objective of the estuary monitoring program is to assist in determining the effectiveness of the implementation of the CCMP. However, this overall objective may encompass several specific monitoring objectives. The identification of these specific objectives begins during *Estuary Characterization.* The characterization process identifies public concerns and formulates a series of corresponding management issues. During characterization, conceptual and predictive models are developed and research results are evaluated to provide a basic understanding of important physical, chemical and biological processes in the estuary. This information is used in the design of the monitoring program to specify a set of variables and ecological processes that can be used to detect changes in the estuary in response to management actions.

Regardless of the scope of the proposed monitoring program, *it is essential to develop explicit statements of the monitoring objectives as well as to establish performance criteria with which to measure monitoring program success.*



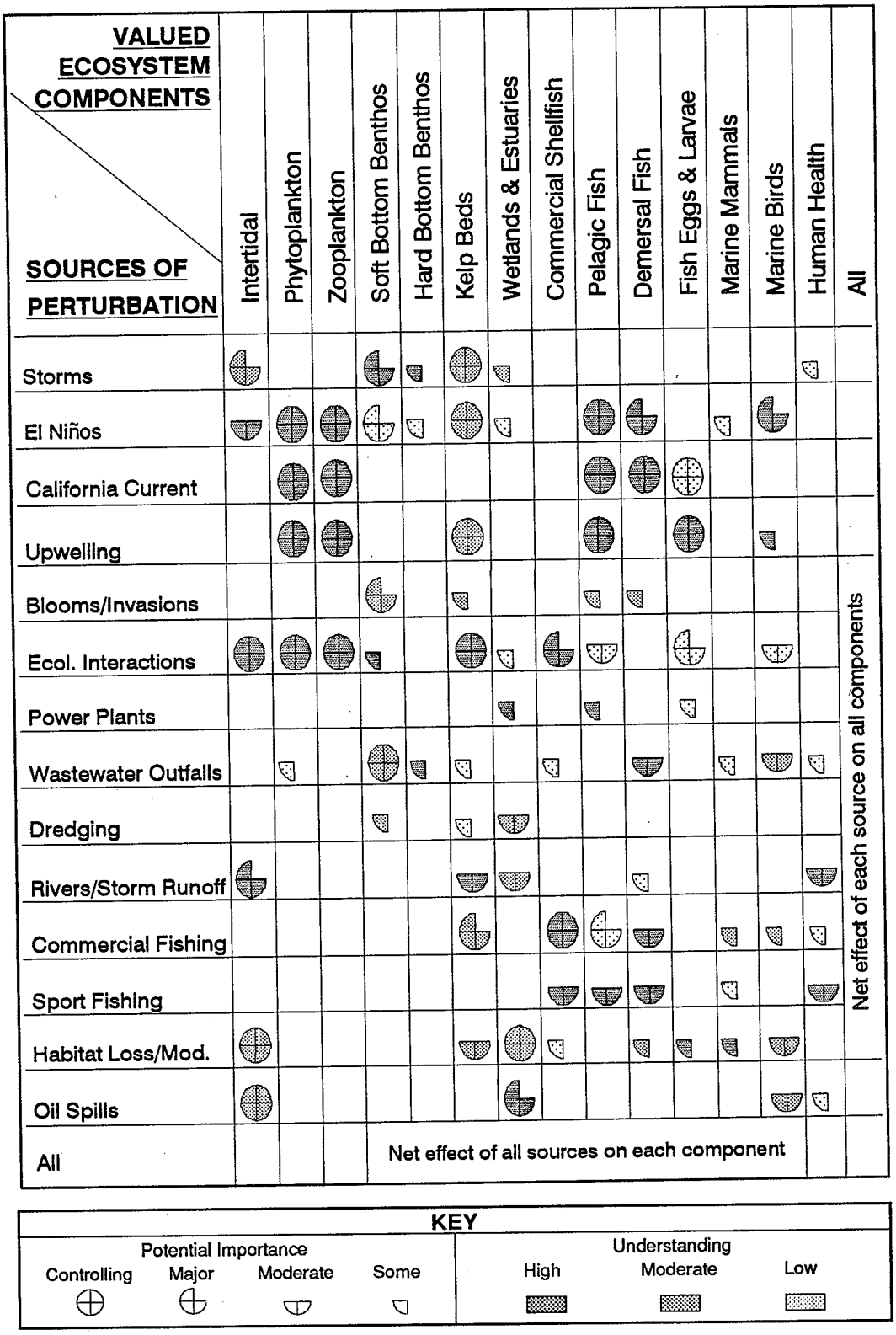
2.1 Monitoring Program Objectives

The development of the monitoring objectives is the culmination of the estuary characterization and the preparation of the CCMP. The characterization process is described in a separate technical guidance document (U.S. EPA, 1992). It involves the identification of public concerns and potential water quality, biological and public health problems in the estuary. A unique approach for identifying these concerns and ecological problems is based on previous work summarized in the National Research Council's *Managing Troubled Waters* (Clark, 1986; NRC, 1990a and 1990b). The key to this approach is the construction of the matrix, shown in Figure 2-1, which identifies Valued Ecosystem Components (VECs) and sources of perturbation. This matrix also summarizes the understanding of the relative importance of each source of perturbation and the degree of scientific certainty associated with knowledge about each impact. Each cell in the matrix summarizes the effects of each perturbation on a single ecosystem component. For example, the information summarized in Figure 2-1 indicates that wastewater outfalls are a controlling factor of soft-bottom benthos communities and that there is a moderate degree of certainty regarding the scientific understanding of the effects of these outfalls in the Southern California Bight. The effects of each identified source of perturbation (e.g., storm water runoff) on all identified resources (VECs) are summarized along a single row. Similarly, each column summarizes the existing knowledge of the impacts on a single resource caused by the complete range of identified sources of perturbation.

This matrix summarizes existing information on the resources of the estuary and potential impacts in an easily accessible manner. The process of developing this matrix also provides an effective tool for building consensus among the wide range of interested parties in the estuary program on the relative priority of monitoring objectives and the different components of proposed monitoring programs. Bernstein *et al.* (1991) describe an Integrated Assessment methodology based on the work by Clark (1986) that guides the selection of monitoring objectives based on the relative importance of the identified resources, the understanding of the underlying controlling processes, and the ability to detect changes in monitoring variables.

Simple conceptual and predictive models developed in the characterization process may also be used to summarize the physical, chemical, geological and biological status of the estuary and identify the factors controlling spatial and temporal changes. Finally, it is necessary to quantify the identified ecological relationships. Existing data should be analyzed to evaluate the strength and direction of identified relationships and to determine the magnitude of uncertainty associated with the existing information. The products of the characterization process should include the identification of the primary management issues. These management issues are used

Figure 2-1. Impacts on the marine environment of the Southern California Bight (Bernstein et al., 1991).



for the development of the CCMP. The CCMP sets environmental quality goals and management objectives for the estuary and specifies actions for achieving these objectives. The monitoring program is designed to verify the predicted results and evaluate the effectiveness of the CCMP implementation, and to recommend corrective actions. When insufficient information is available for estuary characterization and/or CCMP development, a monitoring program may also be designed to fill the information gaps.

Case Study 1 (Appendix A1.0) describes the process that led to the development of the goals of the Puget Sound Ambient Monitoring Program. The essential steps were:

- The development of the Puget Sound Water Quality Management Plan – a comprehensive management plan for Puget Sound and its related waterways. In developing this plan, nine issue papers were prepared. One of these, Comprehensive Monitoring of Puget Sound (PSWQA, 1986), reviewed existing monitoring programs and described the process of developing a comprehensive water quality, sediment, and biological monitoring program.

- During the same period of time, the Office of Puget Sound (EPA Region 10) developed a proposed sound-wide monitoring program which included goals and objectives, sampling design, operation of the program and methods for sampling, analysis and reporting of the data (Tetra Tech, 1986a). This document provided a basis for discussions between members of the Puget Sound Estuary Program (PSEP) technical advisory committee, scientists, agency staff, and other interested parties.

- The Puget Sound Water Quality Management Plan appointed a Monitoring Management Committee to define the goals and objectives of the monitoring program and to modify the monitoring strategy proposed by the Office of Puget Sound. Key aspects of the monitoring program development included workshops that were held for public and scientific peer review, including the Technical Advisory Committee (TAC) of the Puget Sound Estuary Program.

The final report of the Monitoring Management Committee (PSWQA, 1988) summarized the proposed monitoring program and provided explicit statements of the goals and objectives and expected performance. For each monitoring component the rationale, methods, replication and statistical sensitivity, and a listing of appropriate

TABLE 2-1. PUGET SOUND AMBIENT MONITORING PROGRAM DESIGN SPECIFICATIONS FOR SEDIMENT CHEMISTRY SAMPLING (PSWQA, 1988)

Objectives and Rationale

Measuring toxic chemicals of concern will provide data to:

- Assess the potential for sediment toxicity to resident biota.
- Identify areas of Puget Sound that have been, or are, accumulating substantial amounts of toxic chemicals.
- Evaluate temporal changes of toxic chemicals accumulating in sediments.
- Interpret biological and sediment toxicity bioassay data.

Methods

Samples for sediment chemistry will be collected from the upper two centimeters (cm) of sediment, using either a 0.06 m^2 box corer or a 0.1 m^2 van Veen grab. Three grab samples will be taken at each station and composited. The same composite will be used for sediment toxicity bioassays and conventional sediment variables. A minimum of the upper 5 to 10 cm of sediment will be collected for benthic macro-invertebrate abundance determination. Each sampling device has advantages and disadvantages. Although a box corer takes a deeper and possibly less disturbed sample than does a van Veen grab, the box corer is more difficult and more expensive to use. An evaluation of benthic sampling equipment for use in PSAMP is in progress.

Variables to be monitored will include selected EPA priority pollutant metals and selected EPA priority pollutant organic compounds, as well as additional compounds of concern in Puget Sound.

Miscellaneous organic acids and volatile organic compounds will be measured only where a suspected source is present. Intensive surveys conducted by individual agencies under other programs may be triggered by results from this program.

Tributyl-tin has recently been implicated as a human health risk (U.S. EPA, 1985). Studies from other parts of the country have shown accumulations in sediments and animal tissue around large marinas and harbors. The present concern warrants a comprehensive survey for tributyl-tin in sediments and bottom fish tissue in Puget Sound, but it is not included in the monitoring program at this time due to inconclusive sampling results from other parts of the country. Periodic spot checks for this and other contaminants are recommended. Costs of such analyses have not been included in cost estimates for the ambient monitoring program.

Replication and statistical sensitivity

Replicate samples will not be collected for sediment chemistry, thereby precluding statistical analyses among individual stations within a survey. Replicated sampling at all stations was precluded because of the high cost of laboratory analysis and has not been recommended by Puget Sound Estuary Program sampling and analysis protocols. The variability of sediment chemistry estimates will be reduced, however, by the compositing technique recommended. Field and laboratory replication will be required for sediment chemistry samples as part of the quality assurance program. Stations for field replicates will be chosen so as to be representative of certain areas or embayments and sediment types.

Statistical analyses may be performed for related groups (clusters) of stations within a survey or for selected stations over time.

Replicate data from studies on the chemical composition of sediments within the Commencement Bay waterways indicated that coefficients of variation for several groups of organic chemicals ranged from 17-61 percent (Tetra Tech, 1985). Given a coefficient of variation of 30 percent and three to four replicates (in space or time), the minimum detectable difference in mean chemical concentration among stations, at the 95 percent confidence level with a power of 0.8, would be equal to about 100 percent of the overall mean among stations.

Protocols

* Field and Laboratory References: Tetra Tech (1986b, 1986c, 1986d).
* Supporting literature: U.S. EPA (1983), Plumb (1981), U.S. EPA (1982).

sampling protocols were identified. A summary of the design specifications for the sediment monitoring component of the Puget Sound Ambient Monitoring Program is presented in Table 2-1. These design specifications provide an example of PSAMP efforts to clearly state the monitoring objectives and to establish performance criteria. Four major objectives are stated for the sediment chemistry component of the monitoring effort: assess the potential of sediment toxicity, identify toxic accumulation areas within Puget Sound, evaluate temporal changes, and provide supplemental data that will be used to interpret bioaccumulation data and bioassay test results.

2.2 Performance Criteria

The establishment of program requirements, i.e., performance criteria, in terms of the level of precision and accuracy that is necessary to make decisions regarding the success of the monitoring effort will define the expectation of the monitoring program. Issues that must be addressed include: 1) What level of detail will be necessary to make decisions regarding the success of the CCMP? 2) What level of difference must be detected in the monitoring program to initiate modifications in the design and implementation of the monitoring program? This concept of explicitly stating performance criteria is the cornerstone of the systems design approach that is described below. The explicit statement of the monitoring program requirements provides a basis for evaluating expected and actual monitoring program performance (Section 5.0). Evaluation of the effectiveness of alternative monitoring designs also provides a basis for discussion of alternative monitoring approaches and sampling layouts.

Performance criteria are addressed in the design specifications for the sediment chemistry sampling of the Puget Sound Ambient Monitoring Program (Table 2-1). Under the discussion of replication and statistical sensitivity, basic information is provided on background variability and the minimum detectable difference between groups of stations. However, the specification of *performance criteria* associated with monitoring program designs *should be made more explicit* than those presented in these design specifications. For example, one of the stated objectives is to evaluate temporal changes in toxic accumulation. Corresponding performance criteria should be developed by determining the magnitude of trend that can be expected to be detected with the planned level of sampling effort and the period of time that will be required to detect statistically significant trends. The use of historical data and the application of statistical methods to evaluate and establish decision criteria are addressed in Section 5. An example of the use of statistical methods to determine the magnitude of trends that can be measured in a particular monitoring design is presented in Case Study 2 (Appendix A2.0).

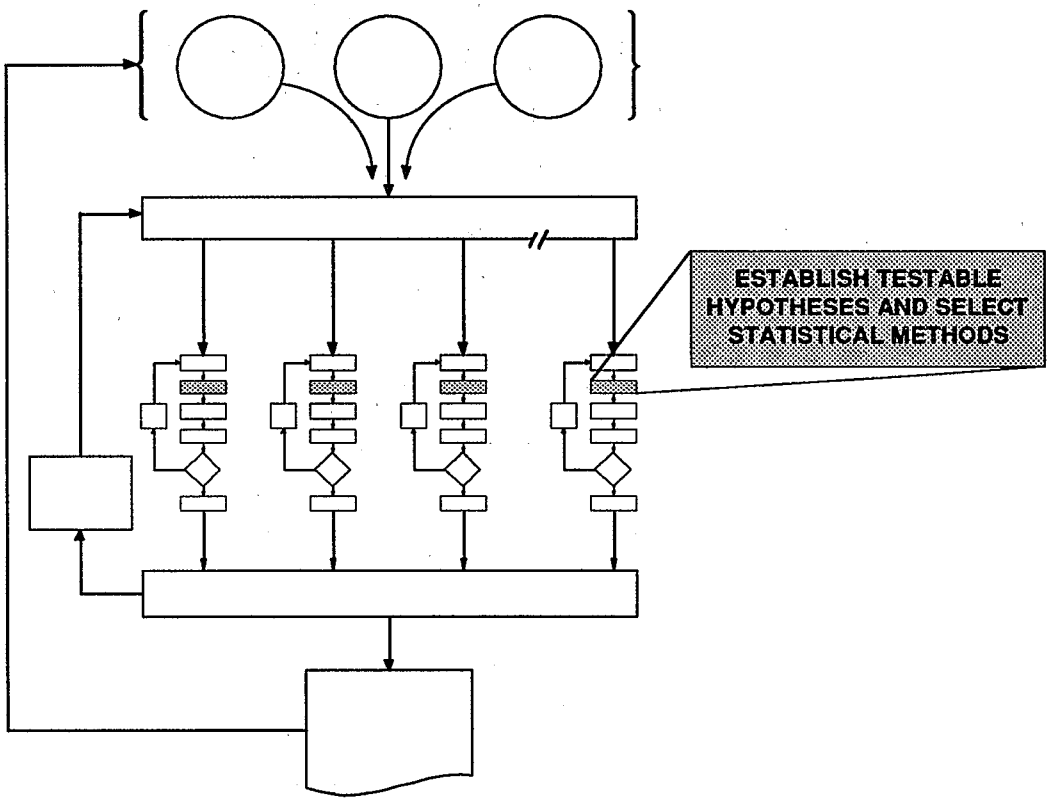
2.3 Additional Guidance

The Quality Assurance Management Staff (QAMS) of EPA has developed an approach to designing data collection programs based on the development of Data Quality Objectives (DQOs). This approach has many of the same elements as the systems approach for designing monitoring programs that are discussed in this document. The DQO process places emphasis on defining the objectives of data collection programs, specification of the decisions that will be made with the data collected, and the possible consequences of the decisions being incorrect (QAMS, 1986). A key aspect to the development of DQOs is the evaluation of the desired degree of certainty in conclusions to be derived from the data. This evaluation is similar to the process described in this document for the development of decision criteria. In both methods, the decision criteria are developed to ensure that adequate information is obtained for making decisions. A case study describing the use of DQOs in Superfund Remedial Investigations is presented by Neptune *et al.* (1990).

3.0 Establish Testable Hypotheses and Select Statistical Methods

Table 3-1 provides examples of monitoring objectives that are common to many NEP monitoring programs. The range of corresponding questions provides some idea of the breadth of monitoring issues that are encompassed in the monitoring objectives.

In designing the monitoring program, these broad objectives must be focused to identify specific monitoring variables and corresponding monitoring activities.



3.1 Establish Testable Hypotheses

The questions identified in Table 3-1 give rise to a number of alternative scientific hypotheses. For example, questions regarding the water quality component of a monitoring program and hypoxia can lead to several hypotheses regarding the response of dissolved oxygen concentrations in the water column. Example hypotheses include:

- Nutrient reduction strategies will result in increased dissolved oxygen concentrations throughout the estuary.
- Any increases in dissolved oxygen concentrations will be long term, improvements will not occur for 20 - 30 years.

It is clear that the monitoring program must be designed to address a wide range of alternative hypotheses. *The recommended procedure for ensuring that sufficient information and the right type of information is developed in the monitoring program is to specify, prior to the collection of any samples, the statistical model that will be used to analyze the resulting monitoring data, and to specify testable (null) hypotheses.*

TABLE 3-1.
EXAMPLES OF MONITORING PROGRAM OBJECTIVES AND ASSOCIATED QUESTIONS

Objective	Questions
Document response of water quality variables to management actions	• Are nutrient reduction strategies effective? • Is the risk of hypoxia reduced? • Is there a decrease in phytoplankton biomass? • Is light transmittance affected? • Is fish community structure affected?
Characterize spatial and temporal patterns in bioaccumulation	• What is the risk of consuming seafood products from within the estuary? • Are there trends in fish and shellfish contaminant concentrations? • Do toxic hot spots exist and what are the influences on bioaccumulation? • What is the relationship between sediment concentrations of contaminants and observed tissue concentrations? • What is the relative contribution of different sources of pollutants?
Monitor the status of the ecosystem	• What are the trends in selected indicators? • What are the consequences of the physiological, morphological and molecular changes on which indicators are based on organism survival and population health? • What is the status of species of commercial and recreational importance?

For example, expanding on the water quality issues above, the process of selecting the statistical model and testable hypotheses for detecting trends in dissolved oxygen concentration is outlined below.

A regression model is used to partition field observations (X_{ij}) between several factors that potentially influence dissolved oxygen concentrations:

$$X_{ij} = \beta_0 + \beta_1 t_i + \beta_2 l_j + \beta_3 t_i l_j + \varepsilon_{ij}$$

where:

X_{ij} = field observation from time i and location j of dissolved oxygen concentration at a specified depth

β_0 = mean of all X_{ij} observations

$\beta_1 t_i$ = temporal component of the measurement

$\beta_2 l_j$ = spatial component of the measurement

$\beta_3 t_i l_j$ = location-time interaction component of the measurement

ε_{ij} = random errors not accounted for by β_0, $\beta_1 t_i$, $\beta_2 l_j$, $\beta_3 t_i l_j$

One possible null hypothesis to be tested in this case is that there is no temporal trend in measured dissolved oxygen concentrations [H_o: $\beta_1 = 0$]. If it is concluded that H_o is false, then an alternative hypothesis [H_a: β_1 is positive (increasing trend) or negative (decreasing trend)] will be assumed to be true. The specification of this model also provides the basis for addressing other scientific hypotheses of interest. For example, is there evidence of location-time interaction [H_o: $\beta_3 = 0$]?

Green (1979) emphasizes the importance of developing testable hypotheses during the design phase of environmental studies and points out that hypothesis formulation is a prerequisite to the application of statistical tests. As pointed out below in Section 5, the development of testable hypotheses and the selection of statistical methods are also the first steps in evaluating the expected performance of the monitoring program. For the dissolved oxygen example, this means determining the minimum trend that can be detected for a specified level of sampling effort. The process of devising testable hypotheses should also be used to initiate discussions between the management committee and technical experts on the importance of individual monitoring objectives

and the relevance of the associated questions. Platt (1964) provides an excellent review of the importance of hypothesis development in the design of scientific investigations in general.

3.2 Selection of Statistical Methods

The statistical tests that will be used to analyze the resulting data must be specified for each hypothesis developed. The applicability of univariate, multivariate, parametric and nonparametric methods must be carefully evaluated. Selected references that provide background information on the use of various statistical methods are summarized in the annotated list presented in Table 3-2. These references will provide program coordinators and scientific staff with a basic understanding of the analytical options available. However, it is essential to involve the statisticians responsible for the analysis of the data in both the development of testable hypotheses and the selection of analytical methods.

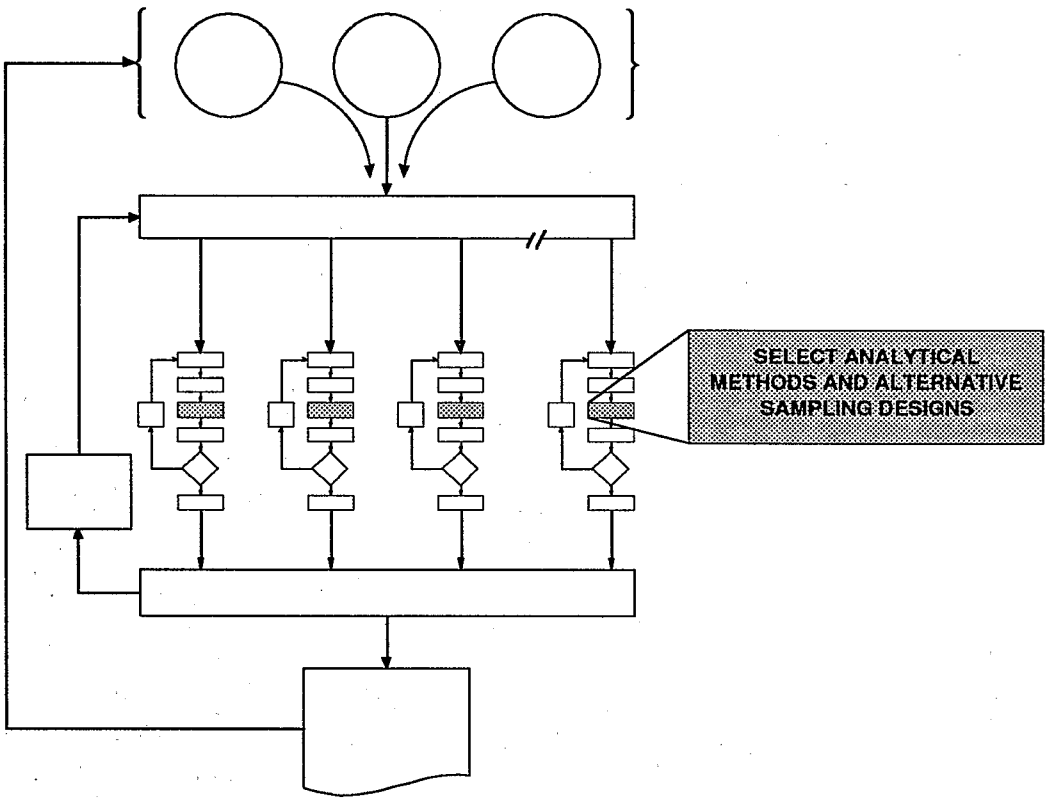
The statistical software that will be used to analyze the data should also be identified at this step in the design process. Berk (1987) describes the attributes of effective microcomputer statistical software. Meads (1990) summarizes the results of a user's survey of six advanced statistical packages available for PCs. *The American Statistician*, a publication of the American Statistical Association, also regularly reviews new statistical software and updates to existing packages.

TABLE 3-2.
REFERENCES TO BASIC MONITORING DESIGN AND STATISTICAL TEXTS

Reference	Description
Monitoring Design	
• Sampling Design and Statistical Methods for Environmental Biologists (Green, 1979)	• Introduction to principles and options for sampling and statistical design. Examples of monitoring design and application of statistical methods.
• Statistical Methods for Environmental Pollution Monitoring (Gilbert, 1987)	• General reference that describes a wide range of statistical methods and their application. Intended for nonstatisticians. Description of several statistical techniques that are not commonly seen in general references.
• Sampling Techniques (Cochran, 1977)	• Comprehensive review of sampling methods and theory. Detailed presentation of topics at an introductory level. Applicability not limited to environmental sampling and monitoring.
• Statistical Principles in Experimental Design (Winer, 1971)	• Description of hypothesis testing concepts.
General Statistics	
• Biometry (Sokal and Rohlf, 1981)	• Basic reference to statistical techniques most frequently used in the biological sciences. Numerous examples of applications.
• Biostatistical Analysis (Zar, 1974)	• Basic reference to statistical techniques most frequently used in the biological sciences. Emphasis on analysis of variance techniques.
• Applied Statistics, Principals and Examples (Cox and Snell, 1981)	• In depth examples of many of the most common statistical applications.
Multivariate Statistics	
• Applied Multivariate Statistical Analyses (Johnson and Wichern, 1982)	• General introduction to multivariate methods.
• Applied Regression Analysis (Draper and Smith, 1981)	• Detailed introduction to regression techniques. Includes section on planning large regression studies.
• Multivariate Statistical Methods: A Primer (Manly, 1986)	• Brief description of the most common multivariate techniques, with examples.

4.0 Select Analytical Methods and Alternative Sampling Designs

The goal of this step in the design process is to develop detailed monitoring program specifications. In addition to the statistical methods, these specifications include the field collection and laboratory analysis methods for individual monitoring variables, and the appropriate quality assurance/quality control procedures. Alternative sampling layouts including numbers and location of sampling points, sample frequency, and the level of sample replication should also be developed. This information will then be used in the next step (see Section 5) to evaluate expected monitoring program performance and to select the most efficient sampling layout among the alternatives.



4.1 Selection of Field and Laboratory Methods

Appendix B of this document provides descriptions of numerous sampling methods that are routinely utilized in estuarine monitoring programs. These descriptions include information on how the data can be used to address the goals of the monitoring programs and to evaluate the success of the CCMP. The essential elements of quality assurance and quality control programs are also described. The purpose of selecting

field and laboratory methods at this stage of the design process is to ensure: the feasibility of using the selected methods in conjunction with the proposed level of sampling effort; that any data used to evaluate expected monitoring performance, a crucial step in the design process, are directly comparable with data that would be collected in the proposed monitoring effort; and, that standardized methods are used. The development and application of an effective QA/QC program is discussed throughout the appendix. The Chesapeake Bay Split-Sample Program (U.S. EPA, 1991) is presented as a valuable approach to maintaining QA/QC.

Standardized protocols or performance criteria should be developed to ensure that the data collected by the different groups participating in the estuary monitoring program are directly comparable. Becker and Armstrong (1988) describe the process of developing standardized sampling and analysis protocols for measuring selected environmental variables in Puget Sound. The key feature of the development of these protocols was a series of workshops at which regional scientists and managers worked together to develop standardized methodologies. The protocols that have been developed are described in the introduction to Appendix B. These protocols were also used as a primary source of information for the description of methods that are included in Appendix B.

4.2 Alternative Sampling Designs

In the development of alternative sampling layouts, consideration should be given to the trade-offs between the benefits of a comprehensive monitoring effort and available funding. In general, federal support for these programs will be limited both in the amount of funding available annually and the duration of funding. Consideration should be given to both limiting the scope of monitoring efforts and making the most efficient use of ongoing monitoring programs in the estuary.

Ideally, the scope of the program should be adequate to meet all identified monitoring objectives. Where the funding is not available to meet all the objectives, however, the individual monitoring components should be prioritized on the basis of the relative importance of related management issues and the availability of existing information. Generally, emphasis should be placed on focusing monitoring efforts in order to attain the level of precision necessary to evaluate the effectiveness of individual management actions, rather than implementing a comprehensive monitoring program that lacks the ability to detect the level of changes expected over time.

Given the wide variety of habitats in individual estuaries, the large variability generally associated with environmental samples, and the limit of funding, alternative sampling strategies should be investigated. Through design optimization, the sampling

effort can be distributed spatially and temporally in such a way as to maximize the amount of information obtained within the area sampled. The strategy behind most sample design optimizations is either to minimize the detectable difference or trend for a fixed cost or to minimize the cost for a specified minimum detectable difference or trend. The strategy adopted will depend upon the specific situation for each monitoring program. In either case, the goal is to obtain the maximum amount of information per dollar spent.

The choice of a sampling design depends on several factors including the objectives of the monitoring program, the type of data that are required to test the null hypothesis, the underlying assumptions of statistical tests, and the spatial and temporal distribution of the monitoring parameters. These factors can affect both the validity of the test results and the efficiency of the monitoring program (cost to obtain a given level of detection). A brief summary of common sampling designs is presented in Figure 4-1.

Sampling Designs

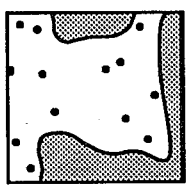
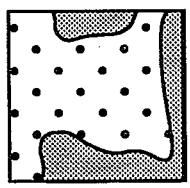
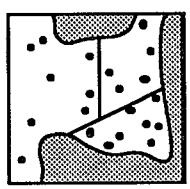
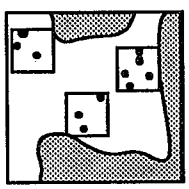
Simple Random:	Samples are independently located at random	
Systematic:	Samples are located at regular intervals	
Stratified:	The study area is divided into nonoverlapping strata and samples are obtained from each	
Multistage:	Large primary units are selected, which are then subsampled	

Figure 4-1. Description of various sampling designs.

The most basic method of collecting monitoring data is simple random sampling. With this design, samples are selected randomly and with equal probability. While this method is easy to implement, there are a variety of sampling designs that can be more efficient. Such designs will produce estimates with smaller standard errors for the same sampling effort, or require fewer samples to obtain the same standard error as would be obtained with simple random sampling.

One such design is systematic sampling. In systematic sampling, sample units are selected at fixed intervals in space or time, usually with a random start. There are many variations of systematic sampling; however, they all share some common advantages and disadvantages. The even coverage obtained with systematic sampling tends to ensure that each sample, on average, is more representative of the population as a whole than a simple random sample. Therefore, such samples tend to have a smaller standard error associated with them. Problems can arise that lead to bias or increased variance if there is an underlying pattern or periodicity in the population over space or time, which is common with environmental data. In addition, it can be difficult to obtain a valid estimate of the standard error if the data cannot be assumed to be distributed randomly.

Another design often used is stratified sampling. By dividing the study area into nonoverlapping homogeneous strata it is possible to optimize the sampling effort in several ways. First, the samples can be allocated to the different strata in proportion to the size of the strata and the variability within the strata, and in inverse proportion to the cost of sampling in those strata. This method will insure that the minimum variance will be obtained for a given cost. Other criteria, such as the ecologic importance of strata and the parameters being measured, can also be taken into account when allocating sampling effort. Stratified sampling also allows the use of the best sampling designs within strata to further increase the sampling efficiency. Stratified sampling works well in a tiered approach because it allows monitoring performance assessment and design modification to be made on a stratum by stratum basis. Stratified sampling also yields estimates for each stratum, providing information that better represents the area being sampled, and is therefore more ecologically meaningful. As an example, two strata may each have a significant trend for a given parameter, but the trends may be in opposite directions. If the data were combined (such as in systematic sampling), the trends might cancel each other out and result in a conclusion of no significant trend. Because stratified sampling ensures that some samples will be taken from each stratum, over the entire study area, it helps ensure that the overall estimate will be more representative on average than one obtained from a simple random sample. This advantage of stratified sampling will be realized even if optimal allocation is not used and the strata are defined arbitrarily with respect to the parameters of interest. In

general, if the variability within individual strata is less than the overall variability in all combined strata, the standard errors obtained with stratified sampling will be less than those obtained from systematic sampling, which will be less than those obtained from simple random sampling. Applications of stratified sampling to environmental studies are found in U.S. EPA (1982a), Jensen (1973) and Reckhow and Chapra (1983).

Multistage sampling is another cost effective method of allocating sampling effort over large areas. In this design, large primary sampling units are composed of smaller secondary units. For large-scale studies, third stage units may also be used. Within each of the selected first stage units, one or more second stage units are selected. In addition to increased sampling efficiency, this method allows intensive sampling to be done only at the second stage, while parameters that are inexpensive to measure can be obtained for the first stage units. This provides some level of monitoring over a wide area, and if problem areas are detected, the distribution of second stage units can be reallocated. The information collected from the first stage units can also be used to implement variable probability sampling at the second stage to further increase the sampling efficiency.

Further information on these sampling designs and methods for calculating required sample sizes and optimal distribution of samples can be found in Gilbert (1987) and Cochran (1977).

4.3 Use of Existing Monitoring Programs

A monitoring strategy that incorporates ongoing monitoring programs or elements from these programs can significantly reduce the cost of the monitoring effort. Existing compliance and resource monitoring programs may produce data that can completely satisfy, or augment, the spatial and temporal coverage required by an NEP monitoring program. Additionally, by adopting sampling and analytical methods of ongoing monitoring programs as standard protocols for NEP monitoring program components, data from these existing monitoring programs may be used in evaluating the effectiveness of the CCMP.

An inventory and evaluation of existing federal, state, local, and volunteer monitoring programs within the estuary drainage basin should be conducted in order to assess their usefulness and applicability in evaluating the effectiveness of the CCMP. Key tasks of this assessment process include:

- Identification of existing and planned programs as well as special projects that may contribute data useful in evaluating the effectiveness of the CCMP

- Determination of whether NEP monitoring program objectives could be cost-effectively met by incorporating sampling and analytical methodologies from these existing monitoring programs

Specific monitoring variables, statistical and analytical methods, and quality assurance/quality control (QA/QC) protocols selected will depend upon the stated objectives and action plans outlined in the CCMP, and specific hypotheses to be tested in the monitoring program. Therefore, techniques from existing monitoring programs may not always be adopted. However, whenever possible, it is recommended that programs work to ensure comparability of their methods with existing programs.

Several specific opportunities for taking advantage of ongoing monitoring efforts are described below.

NOAA's National Status and Trends (NS&T) Program

NOAA's National Status and Trends Program is designed to assess the current conditions of environmental quality in the nation's coastal zone and to determine whether these conditions are improving or deteriorating (NOAA, 1989). In order to achieve this objective, NOAA currently supports the Benthic Surveillance Project and the Mussel Watch Program.

The Benthic Surveillance Project regularly measures concentrations of contaminants in sediments and tissues of bottom-dwelling fish. The occurrence of external and internal symptoms of disease (e.g., fin erosion and liver tumors) are also documented. The Benthic Surveillance Project currently collects and analyzes sediment and bottomfish samples at 75 estuary sites.

The Mussel Watch Program collects mussels and/or oysters once a year from approximately 200 sites nationwide (NOAA, 1989). Analyses of sediment and bivalve tissue concentrations of trace metals, DDE, PCBs, aromatic hydrocarbons, and radionuclides are conducted.

NS&T has developed standardized methods for analysis, quality assurance (QA), and quality control (QC) for fish and shellfish tissue and sediment contaminants. The methods employed in this program and sampling locations should be reviewed to

determine the feasibility of their incorporation into the planned monitoring efforts in each estuary. For detailed information concerning analytical and QA/QC methods, contact:

NOAA National Status and Trends Program
NOAA N/OMA32
6001 Executive Blvd
Rockville, MD 20852

In addition, NOAA is establishing a National Estuarine Reserve Research System (NERRS). National Estuarine Research Reserves are established and managed for long-term environmental monitoring and scientific research. The results of scientific research at the Reserves are important sources of information and data to support coastal zone management and decision making. For more information concerning the NERRS, contact:

Marine and Estuarine Management Division
Office of Ocean and Coastal Reserve Management
NOS/NOAA
1825 Connecticut Avenue, NW
Washington, D.C. 20235

EPA's Environmental Monitoring and Assessment (EMAP) Program

The goal of EMAP's Near Coastal (EMAP-NC) Program is to monitor the condition of near coastal ecosystems, evaluate the relationship between ecological condition and anthropogenic disturbance, and assess the effectiveness of pollution control actions and environmental policies on a regional and national scale. EMAP will provide information on biological and chemical indicators, sampling design and methods, analytical methods, and QA/QC protocols.

EMAP-NC sampling design consists of three schemes (U.S. EPA, 1990b). EMAP's regionalization scheme divides the nation's estuaries and coastal resources into bio-geographical provinces (e.g., Virginian, Carolinian, Louisianian, Acadian, Columbian, and Californian provinces). EMAP's classification scheme classifies estuaries into three resource classes that have similar physical features:

- Large, continuously distributed estuaries (e.g., Chesapeake Bay, Long Island Sound)

- Large, continuously distributed tidal rivers (e.g., Potomac, Delaware, Hudson Rivers)

- Small, discretely distributed estuaries, bays, inlets, and tidal creeks and rivers (e.g., Barnegat Bay, NJ; Indian River, FL; Lynnhaven Bay, VA)

EMAP's sampling scheme consists of elements of systematic, random, and fixed location sampling. Large, continuously distributed estuaries are sampled using a randomly placed systematic grid, with grid points about 18 km apart. Large tidal rivers are sampled along systematically spaced lateral transects. Transects are located about 25 km apart. Two sampling points are located on each transect, one randomly selected, and one selected using scientific judgement to identify sampling locations that may be indicative of degraded conditions in the system. Small estuaries are sampled by partitioning them in groups of four, selecting one estuary randomly from each group of four, and sampling at two stations in each small estuary selected. EMAP will operate on a four year sampling cycle, with one-fourth of the sites in a region sampled each year. Sampling will be undertaken only during the months of July and August. Given this sampling design and schedule, it is clear that a very small amount of EMAP data, if any, is likely to be available to most NEP estuary programs in a particular year. The stratified random sampling approach developed by the EMAP program may be of use to some NEP estuary programs if applied on a much smaller scale. The EMAP program is currently evaluating the advantages of sampling on alternative spatial scales.

EMAP-NC indicators of environmental quality are described in EMAP's Near Coastal Program Plan for 1990 and Ecological Indicators documents (U.S. EPA, 1990b and 1990c). Ecological indicators include:

- Response indicators
  - Benthic species composition and biomass
  - Gross pathology of fish
  - Fish community composition
  - Relative abundance of large burrowing shellfish
  - Histopathology of fish
  - Apparent redox potential discontinuity

- Exposure indicators
  - Sediment contaminant concentration
  - Sediment toxicity

- Contaminants in fish flesh
- Contaminants in large bivalves
- Water column toxicity
- Continuous and point measurements of dissolved oxygen concentration

- Habitat indicators
  - Salinity
  - Sediment characteristics
  - Water depth

- Stressor indicators
  - Fresh water discharge
  - Climate fluctuations
  - Pollutant loadings by major category
  - Land use patterns of watershed by major category
  - Human population density/demographics
  - Fishery landings statistics

Methods for collecting many of these indicators are addressed in the methods section (Appendix B).

The information from EMAP-NC is intended to establish ecological trends over the time frame of decades. Although this information may not be immediately available to the National Estuary Program, EMAP-NC does provide valuable information and assistance in the areas of sampling design, indicators, sampling methods, quality assurance and information management. Additionally, EMAP-NC is implementing a secondary monitoring strategy that focuses on local and state issues. The objective is to foster the incorporation of EMAP-compatible monitoring efforts for near-coastal resources into sampling by state and local agencies. Such efforts are intended to build upon the present EMAP design to permit assessment of environmental conditions at spatial and temporal scales not presently addressed by EMAP.

For detailed information concerning EMAP analytical, statistical, and QA/QC methods, consult the Director of EMAP's Near Coastal Waters Monitoring Program at:

Environmental Research Laboratory
U.S. Environmental Protection Agency
27 Tarzwell Drive
Narragansett, RI 02882

Local Monitoring Programs

In addition to ongoing compliance and resource (e.g., fisheries) monitoring that is conducted in each estuary, there are also other opportunities to augment the estuary monitoring programs. For example, states are required under 305(b) of the Clean Water Act to conduct water quality assessments, and they are encouraged to include assessments of trends in their reports that are submitted biennially to EPA (U.S. EPA, 1989b). Other monitoring programs are conducted by state agencies, universities and pollutant dischargers. These programs should be evaluated to determine their use in monitoring CCMP implementation.

State sponsored and private volunteer monitoring programs have been shown to be very valuable in collecting data on estuarine water quality, beach litter, marine mammal strandings, and the status of other estuarine resources (Armitage *et al.*, 1989). Volunteers in the Chesapeake Bay watershed have collected data used to help verify water quality models and to identify correlations between measured variables, such as low dissolved oxygen, and the frequency of observed events such as fish kills and algae blooms (Ellett, 1988).

Volunteer monitoring programs have also been useful in developing an educated and involved constituency committed to protecting water resources (U.S. EPA, 1990d). Partnerships between the public and government agencies responsible for management must be developed if National Estuary Program CCMPs are to be effectively implemented. Establishment of volunteer monitoring programs has proven to be an effective way to build public commitment to achieving environmental quality goals and objectives in the CCMP. Through participation in monitoring programs, citizens learn how they contribute to pollution problems and develop a sense of stewardship toward the waters they are monitoring. Volunteer monitoring programs also help the public to understand the difficulties faced by the scientific community in linking water quality changes to impacts on living resources.

Each National Estuary Program Management Conference should establish a volunteer monitoring component as an integral part of its monitoring program. The experience of citizen monitoring programs throughout the country proves that volunteers can be trained to carry out a wide variety of environmental monitoring tasks, provided they are given the appropriate equipment and instruction. In estuaries, volunteers can be especially helpful in upstream areas not normally covered by a state's monitoring network. Basic water quality measurements such as pH, transparency, salinity, dissolved oxygen, and temperature can provide useful information to the comprehen-

sive monitoring program. Trained volunteers can also be used to assess aquatic vegetation in the estuary and can provide information on acute problems such as spills, fish kills, and algae blooms. EPA has published a guidance document describing how to plan and manage effective volunteer environmental monitoring programs (U.S. EPA, 1990d). The document provides an overview of the use of citizen volunteers in environmental monitoring. It discusses how to plan and organize volunteer monitoring projects, how to involve the media, and how to prepare quality assurance plans for volunteer programs.

In general, estuary monitoring programs must select cost-effective methods that provide data essential to assessing the effectiveness of the CCMP. Whenever possible, programs should work to ensure comparability of their methods with applicable ongoing programs. Key aspects of the incorporation of existing state agency and volunteer monitoring programs into the overall design for the Puget Sound Ambient Monitoring Program are described in Case Study 1 (Appendix A1.0).

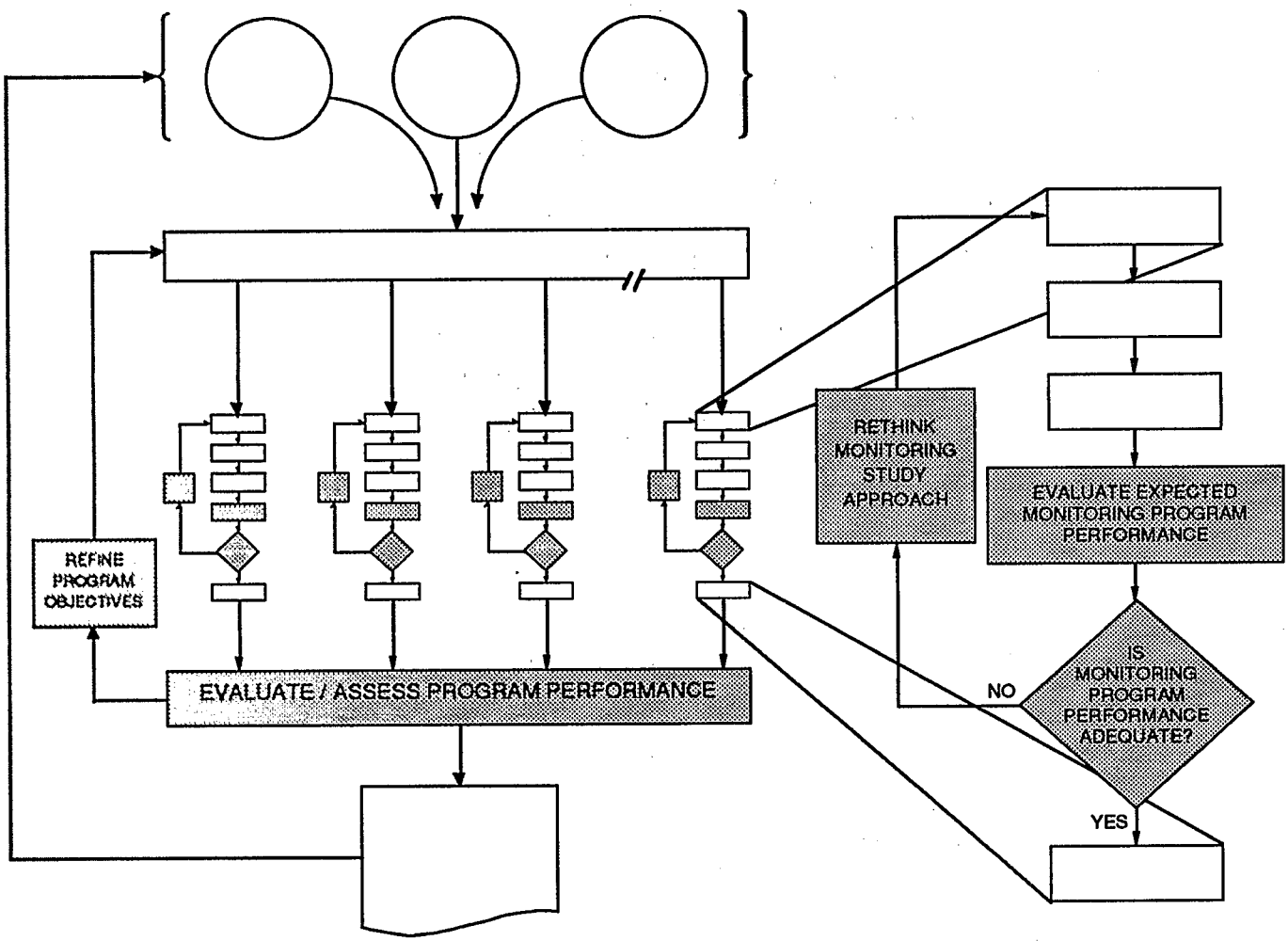
5.0 Evaluate Monitoring Program Performance

Although often overlooked and neglected, the evaluation of monitoring program performance is potentially the most important step in the design and review process. The performance evaluation motivates the development of explicit statements of program objectives as well as the specification of quantitative performance criteria during the design phase. During the course of the monitoring effort, performance evaluations provide a systematic procedure for measuring success in terms of the ability to meet program goals. The periodic evaluation process also identifies the need to modify sampling design and methods.

Evaluation procedures are essential because the information developed in the monitoring programs must be sufficiently precise and scientifically defensible. The monitoring programs will provide the primary source of information that will be used to evaluate the success of the CCMP. This information will be used as a basis for determining the efficacy of selected management strategies and the accuracy of model predictions upon which many management decisions have been based. The monitoring programs will also provide quantitative information that will guide decisions regarding needed modifications to the management plan.

Additionally, the cost of the estuary monitoring programs will be substantial. In order to protect this investment, *it is essential to assess expected performance prior to collecting the first samples.* This performance information will provide the basis for determining the feasibility of proposed sampling strategies, selecting the most effective monitoring components and variables, and optimizing the overall monitoring effort.

The two types of performance evaluations are shown in Figure 1-1 and highlighted in the schematic on the next page. The first is the evaluation of the *expected performance of individual components* of the monitoring program (e.g., the evaluation of trends in toxic chemical accumulation in sediments). The first evaluation takes place during the design phase. The second type of performance evaluation is the *assessment of overall program performance.* This assessment takes place after the monitoring program has been implemented (e.g., after the first full year of data have been collected). The objective is to determine if the overall goals of the program are being met by the individual monitoring components and if the program should be modified by adding, deleting or expanding the scope of individual monitoring components. The essential feature of both types of evaluation is the existence of a feedback loop that provides the pathway for modifying the system's design based on monitoring program performance.



5.1 Evaluate the Expected Performance of Individual Monitoring Program Components

The establishment of performance criteria (e.g., the ability to detect a change in chlorophyll concentrations of 5 µg/ℓ over a period of five years) is a fundamental part of developing monitoring objectives. As indicated in Section 2.0, these performance criteria represent the level of change that must be detected in order to make management decisions regarding the effectiveness of the CCMP. It is these performance criteria that will be used to evaluate the applicability of individual components of the monitoring program. The specification of sampling methods for proposed monitoring program components described in Step 3 (Section 4.0) includes the development of alternative sampling strategies, including the monitoring variables/indicators and the level of sampling effort (numbers of sampling stations and sample replicates). The goal of the performance assessment is to evaluate the effectiveness of these alternative sampling designs in terms of the established performance criteria. The results will

provide the basis for determining the relative benefits of individual monitoring components and selecting the final monitoring design.

The questions that will be addressed in these analyses are:

- Can the proposed sampling effort meet the needs of the monitoring program as defined in the performance criteria associated with the stated objectives?

- How can the proposed program be modified to ensure that these objectives are met?

As indicated by the feedback loop shown in Figure 1-1, this is an iterative process. Proposed sampling designs are evaluated and modified, if necessary, to meet the overall objectives. The tools for conducting these analyses are described in Section 5.3.

5.2 Evaluate Overall Program Performance

The overall performance of the monitoring program should be evaluated at periodic intervals. Initially, this evaluation should take place at the conclusion of the first year of sampling. This evaluation should compare the results with the expected monitoring performance, and a list of required modifications should be prepared. Opportunities for streamlining the program should be identified, and the performance criteria should be reviewed and revised, if necessary, for subsequent evaluations.

5.3 Statistical Power Analysis Methods

The primary tool for conducting these analyses is statistical power analysis. Statistical power analysis provides an evaluation of the ability to detect statistically significant differences in a measured monitoring variable. The importance of these analyses can be seen in the examination of the possible outcomes associated with testing the null hypothesis (e.g., H_o: sampling location has no effect on observed sediment contaminant concentrations) shown in Figure 5-1:

- The null hypothesis is true, and it is rejected. This is referred to as a Type I error (α) and is commonly called the significance level of the test. By convention this value is routinely set at 0.05, i.e., the investigator accepts a small probability of incorrectly concluding that there are differences in sediment contaminant concentrations between sampling locations. Lower or higher values of the significance level of the test may be appropriate depending on the consequences of incorrectly rejecting a true null hypothesis.

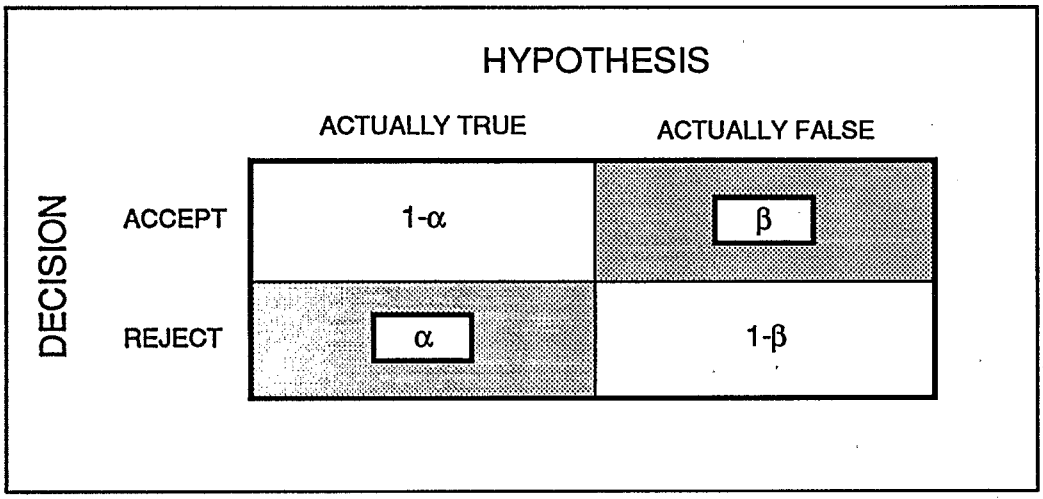


Figure 5-1. Hypothesis testing: possible circumstances and test outcomes.

- The hypothesis is true, and it is accepted. This is the complement of the Type I error (1- α).

- The hypothesis is false, and it is accepted. This is referred to as a Type II error (β). While the significance level of the test is consistently reported with the results of statistical tests, the probability of accepting the null hypothesis when it is not true (Type II error) is almost never reported with statistical test results. Moreover, the consequences of the Type II error are probably not fully comprehended by many investigators.

- The hypothesis is false, and it is rejected. This is the complement of the Type II error (1- β) and is referred to as the power of the test. Therefore, *statistical power is the probability of correctly detecting an effect.*

Consideration of these possible outcomes of a statistical test leads to three important conclusions. The first is that failure to reject the null hypothesis does not justify its acceptance. For example, the failure to reject the null hypothesis (e.g., that location has no effect on sediment contaminant concentrations) may occur either because there really is no effect (probability = 1 - α) or because the power of the test is so low (probability = β). In the later case, *a Type II error occurs because the statistical test is weak.* This may be due to the highly variable nature of the sampling environment or the low level of sampling effort. In either case, it is possible to evaluate the probability of the Type II error for any statistical comparison of environmental data. Therefore, the second conclusion is that the probability of the Type II error should be reported with all statistical test results. The third conclusion is most relevant to the

design of the NEP monitoring programs – the expected power of the statistical test should be evaluated prior to implementing the sampling program.

Although statistical power analyses are not routinely conducted with statistical tests of significance, there is ample guidance available and the tests can be easily performed. Power analyses can be conducted using standard equations and tables or nomographs. The basic tools for conducting these analyses are provided by Cohen (1977), Dixon and Massey (1969), and Winer (1971). More comprehensive tables and nomographs are provided by Pearson and Hartley (1951), Tang (1938), Lehmer (1944), and Scheffe (1959), but the use of these references requires more advanced statistical training. There are also a wide range of computer programs available for conducting statistical power analyses. Goldstein (1989) reviewed several MS/PC-DOS power analysis programs and compared the methods they cover, their ease of use, graphics capabilities and their computational accuracy. Additionally, a statistical power analysis tool for analysis of variance is available on the EPA's Ocean Data Evaluation System (U.S. EPA, 1987a), and the U.S. EPA guidance for conducting fish histopathology studies (U.S. EPA, 1987b) provides nomographs for the power analyses associated with contingency table analysis.

Most basic statistical texts address hypothesis testing and the concepts of statistical power analysis. The statistical texts by Dixon and Massey (1969) and Winer (1971) provide introductory level descriptions as well as the tables for conducting these analyses. The statistical text by Scheffe (1959) provides a theoretical description of power tests for the analysis of variance.. The Technical Support Document for the ODES Statistical Power Analysis Tool (U.S. EPA, 1987a) provides a detailed introductory level description of power tests for the analysis of variance.

The power of all statistical tests is dependent upon the following design parameters:

- significance level of the test (α)
- level of sampling effort (i.e., number of sampling stations and sample replicates)
- minimum detectable difference in the effect that can be detected
- natural variability within the sampling environment

This relationship between the power of a statistical test and the design parameters makes several types of power analyses possible. The power of the test can be determined as a function of any of these design parameters. Alternatively, the value of any individual design parameter required to obtain a specified power of a statistical test can be determined as a function of the other parameters.

The results of statistical power analyses are generally reported in two formats. In the first, shown in Figure 5-2, the minimum difference in the effect that can be detected is shown as a function of level of sampling effort (number of replicate samples). The results of this type of analysis provide a quantitative comparison of alternative sampling layouts, and they are especially useful in the evaluation of proposed NEP monitoring programs. Using these results, the level of sampling effort required to obtain a selected level of precision in the monitoring program can be determined. This example was taken from the evaluation of bioaccumulation monitoring strategies (U.S. EPA, 1987c). Historical data for liver concentrations of PCBs in winter flounder were used to evalu-

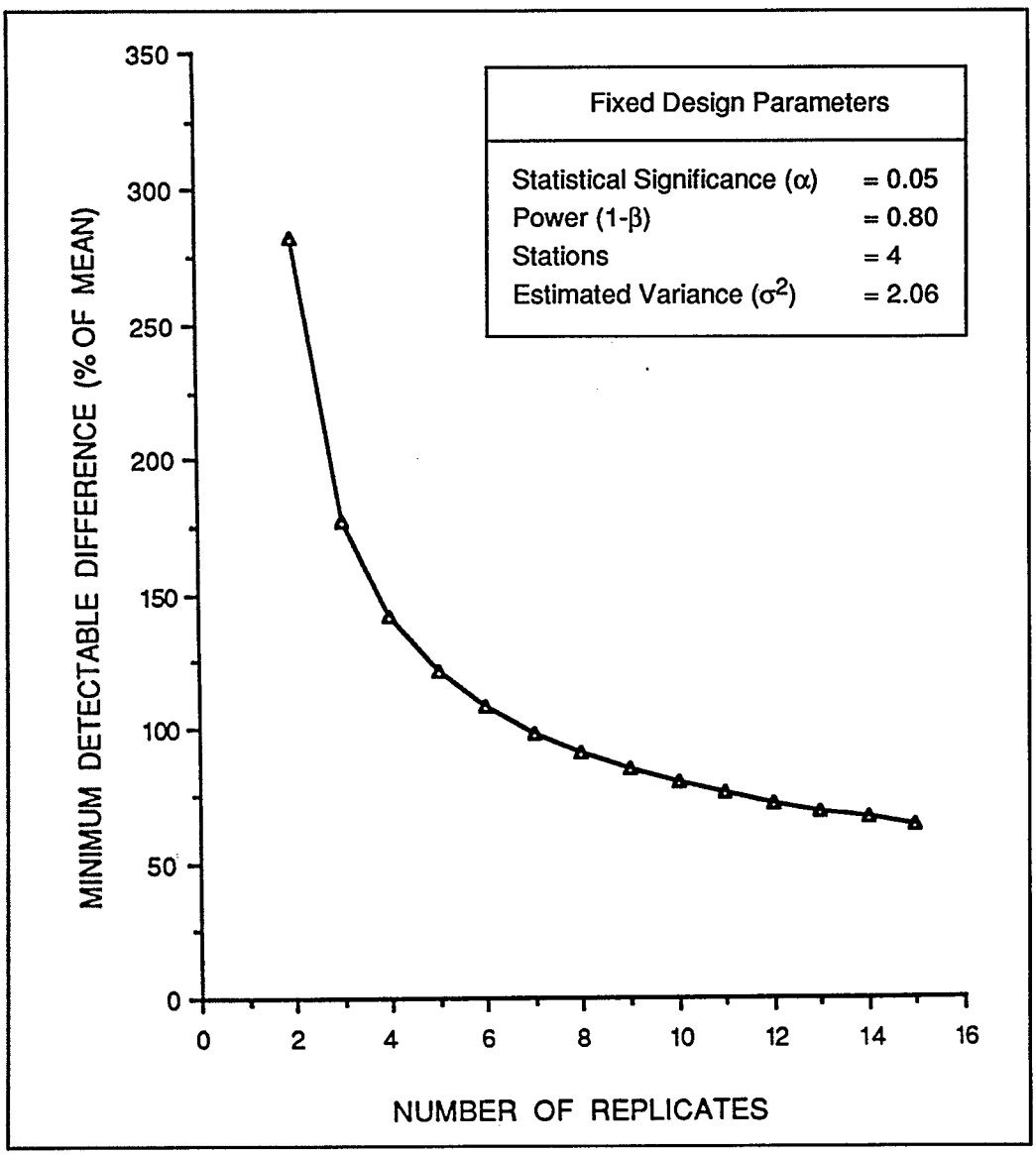


Figure 5-2. Minimum detectable difference vs. number of replicates for fixed set of design parameters.

ate the expected performance of alternative sampling designs. The results shown in Figure 5-2 were taken from the first phase of the analyses. They indicate that the minimum difference in tissue concentrations of PCBs that could be detected between sampling locations with the collection and analysis of tissue from five fish at each location was approximately 120 percent of the overall mean at all stations, or 4.9 mg/kg. Additionally, these results also show that the sampling program would require the collection and analysis of tissue from seven fish at each location to detect a difference equal to the overall mean among sampling locations. Based on the results of these early analyses during the design phase of the monitoring program, alternative sampling strategies were evaluated. Additional power analyses were conducted to evaluate the compositing of fish tissue prior to laboratory analyses. The results indicated that the collection of replicate composite samples would permit the detection of substantially smaller differences in tissue concentrations of PCBs at a much lower cost.

Statistical power analyses may also be displayed as shown in Figure 5-3. The power of the test (the probability of detection) is shown as a function of the minimum detectable difference that can be detected between locations or over time. These results, taken from Case Study 2 (Appendix A2.0), were obtained from the analysis of a subset of water quality data that were collected in Chesapeake Bay. As part of the 1987

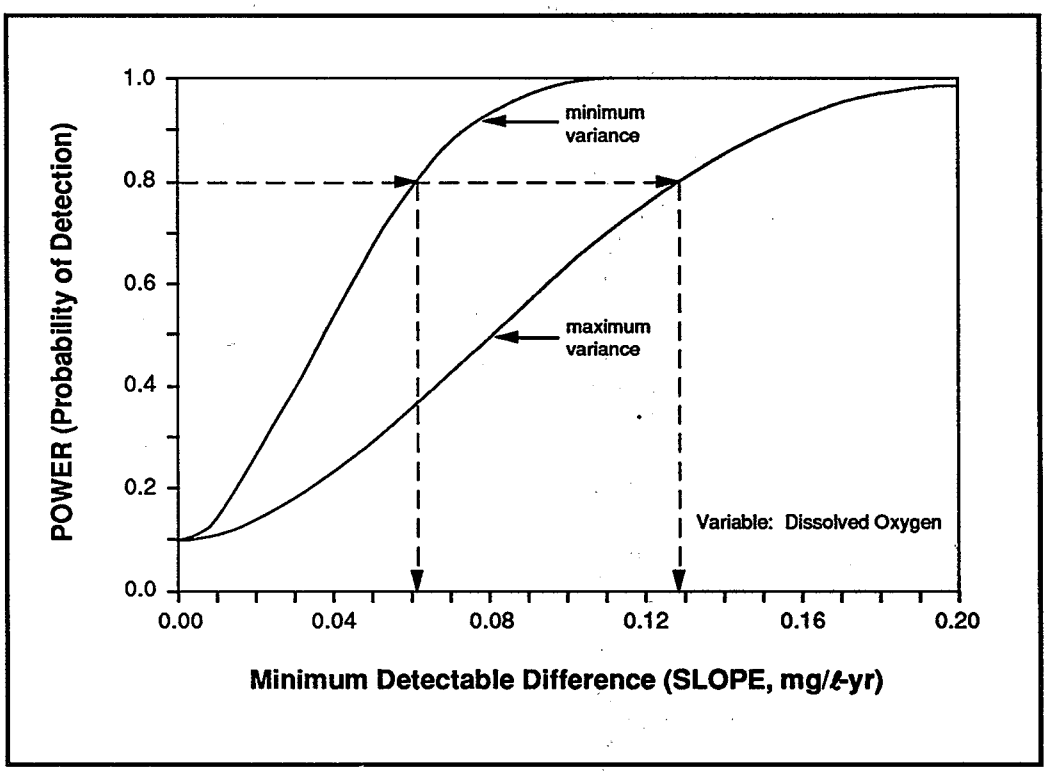


Figure 5-3. Power vs. minimum detectable difference.

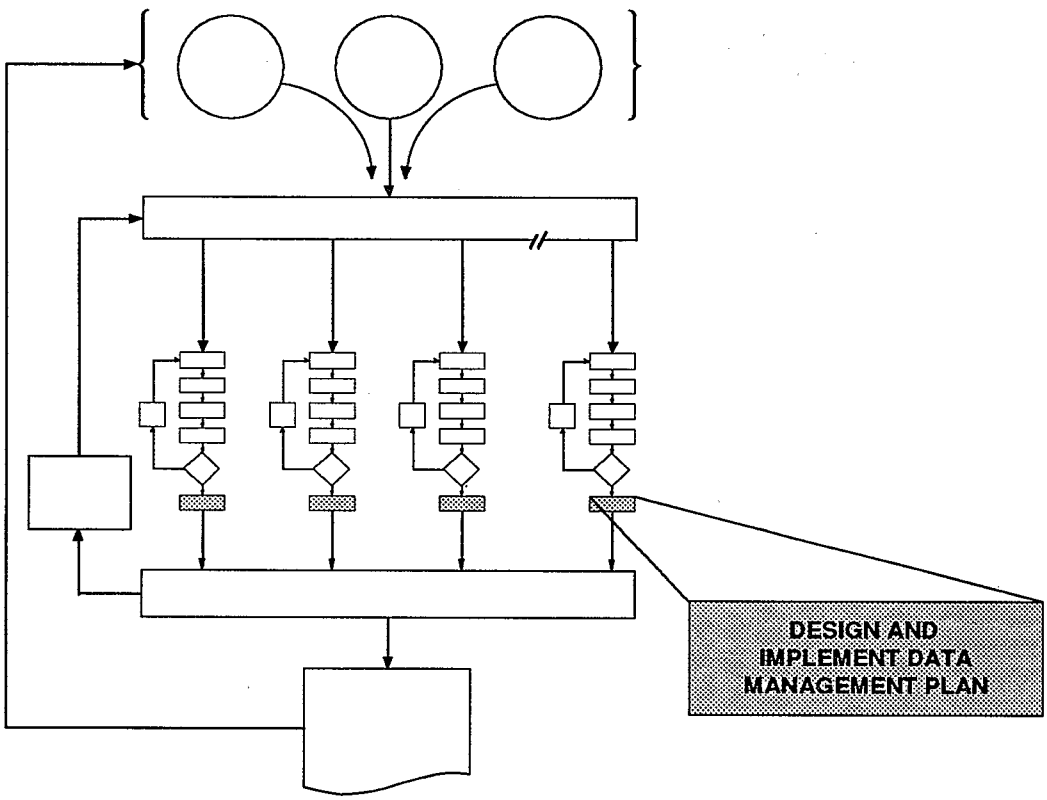
Chesapeake Bay Agreement, a commitment was made to reduce the total inputs of nitrogen and phosphorus entering the mainstream of the Chesapeake Bay by at least 40% by the year 2000. The target of 40% nutrient reduction was developed by modeling the environmental conditions in the Chesapeake Bay with a two-dimensional, steady-state model. The modeling results indicated that, after full implementation of the 40% reduction goal, the lowest average dissolved oxygen would be 1.6 mg/ℓ and no waters would be anoxic. Therefore, the minimum performance criterion for the monitoring program should be the ability to detect a difference in dissolved oxygen equal to 1.6 m/ℓ.

To test the ability of the existing monitoring program to meet the performance criterion, historical data were used to estimate measurement variability. Statistical power analyses were then conducted, using estimates of the maximum and minimum variance. The results shown in Figure 5-3 indicate that the minimum trend in dissolved oxygen concentration that can be detected with a probability of 0.80 and ten years of data is on the order of 0.06 to 0.13 mg/ℓ-yr. These results indicate that the existing monitoring program would meet the specified performance criterion.

The relationship between power and minimum detectable difference, shown in Figure 5-3, provides the information required to evaluate the probability of a Type II error and the probability of detecting specific levels of effects in a proposed sampling program. Recent examples of the application of this type of power analysis include: Parkhurst (1985); Toft and Shea (1983); and Rotenberry and Wiens (1985). The necessity for this type of analysis in the evaluation of statistical test results is provided by Peterman (1989).

6.0 Design and Implement Data Management Plan

Data management and data analysis, two key components of the monitoring study that are often overlooked in the design of monitoring programs, are as important to the success of the monitoring effort as the collection and laboratory analysis of field data. Moreover, the cost of effective data management/data analysis can be substantial. On the order of 20 percent of the budget allocated for the monitoring program should be reserved for data management and data analysis activities. Failure to plan for these costs can result in the loss of information due to inadequate data preservation and limited analysis of the monitoring data that are collected.



Recent characterization efforts conducted by individual estuary programs, at considerable expense, found that historical data are not readily available and that essential quality assurance information necessary to evaluate the comparability of data sets is often not preserved. Generally, it was found that although large expenditures are often made on data collection, the amount of funding allocated to data management and data analysis is relatively small and inadequate.

6.1 Data Management

The need to assimilate and integrate historical information, as well as planned monitoring efforts, should drive the development of a data management strategy. Failure to plan for data management can result in the loss of the information due to inadequate data preservation.

The development of a data management strategy must consider the following questions:

- Where will the data go?
- How will these data be stored?
- Who will maintain the data base?
- How will data be checked and loaded into the data base?
- How accessible will the data be?
- Will statistical, graphical, and report generating tools be available?
- How much will it cost?

A computer system will be essential for the management of the data collected by the estuary monitoring programs. It should be operational prior to implementation of the monitoring program and should have the following attributes:

- Centralized storage of raw data
- Easy access and use
- System documentation
- Quality assurance procedures
- Linkage to graphical, statistical and report generation routines
- Long term availability and flexibility

A centralized data source provides the ability to transfer data between investigators and to conduct analyses that utilize data from different monitoring program compo-

nents. An additional feature of a centralized data management approach is the ability to designate a system administrator who has responsibility for system documentation and data quality assurance.

The quality assurance information that must be reported with each data set must be defined prior to implementation of the monitoring effort. The objective is to identify key field, laboratory and quality assurance information that would allow future users of the data to make informed decisions regarding the comparability of historical data sets. This set of basic reporting requirements should be developed for all data types collected. The Ocean Data Evaluation System (ODES), supported by EPA's Office of Water, and the data system developed for the Puget Sound Ambient Monitoring Program provide good models for the implementation of data quality assurance procedures. Each data set that is submitted to ODES, for example, undergoes an extensive quality assurance review, and the results of this review are summarized in a report that is accessible from within the system. Extensive documentation for ODES is available (U.S. EPA, 1987d and 1988b).

The data management system that is adopted by the estuary programs must also provide basic graphical, statistical and report generation capabilities and/or the ability to download data easily to data analysis packages. It must also be flexible enough to address new data types and analytical needs. Finally, a long term financial commitment must be made to the system in order to establish user confidence in the ability to store and access data over a long time period.

The NEP has developed a data management policy to ensure that all potential users, both inside and outside the NEP, have access to environmental data generated under the program. Responsibility for identifying and selecting data management support remains with the NEP Management Conferences. The selection of a data management system by each program should be based on an evaluation of characterization and monitoring requirements for the estuary. Use of existing systems, where possible, is encouraged.

6.2 Data Analysis

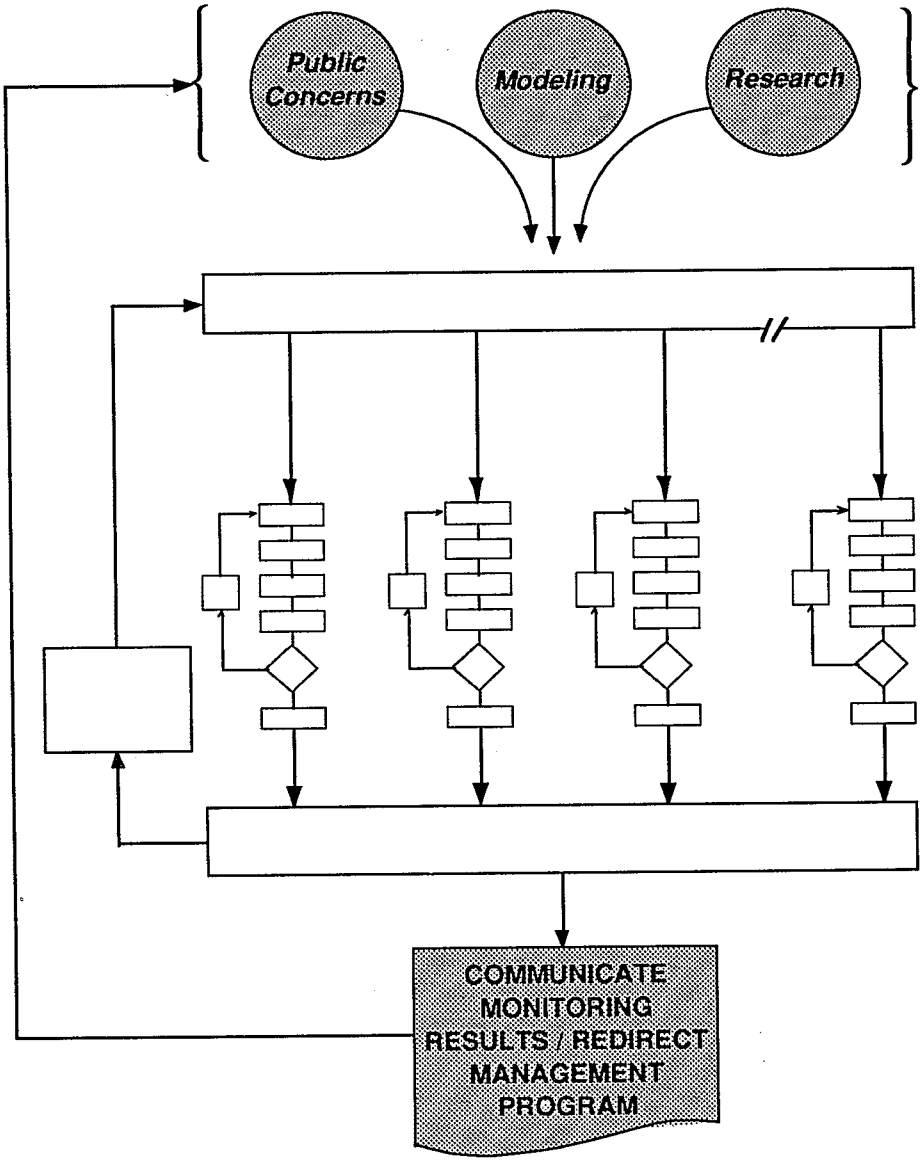
As indicated in Section 5.0, an essential element of the monitoring plan will be the specification of a timetable for analyzing the data and assessing monitoring program performance. The assessment of monitoring program performance should be used to refine monitoring program objectives and modify individual monitoring program elements to satisfy these objectives. Initially, monitoring program evaluations should be conducted after the first year of data collection. Subsequent interim evaluations should be conducted at two or three year intervals.

The primary purpose of other data analysis activities will be to test the hypotheses developed in Step 2 of the design process (see Section 3.0). Additional goals are to summarize the data, generate new hypotheses, and evaluate the uncertainty associated with the measurements and conclusions. Additional analyses should be designed to produce information for use by groups with diverse technical backgrounds.

A wide range of statistical and graphical tools are readily available for use in meeting these goals. Recently, there has been an increased interest in the development of Geographical Information System applications for graphical analysis and information display. The National Estuary Program is currently funding projects to demonstrate the use of this tool to synthesize a broad range of data collected by the estuary programs, and to effectively communicate this information to interested groups.

7.0 Communicate Program Results

One of the primary goals of the monitoring program is to provide information that can be used to redirect and refocus the CCMP. To achieve this goal, emphasis should be placed on distributing the data that are collected in the estuary monitoring programs. The data collected by individual investigators should be made readily available to the scientific community for comparative studies that relate information from different components of the program. Section 6.0 discusses the need for the development and implementation of an effective data management strategy. Emphasis should also be placed on the analysis of these data. The dissemination of the recorded monitoring data is not a sufficient mechanism for communicating the monitoring results. Statisti-



cal analysis of the monitoring data is essential, and graphical and written summaries should be produced for agency managers charged with implementing the CCMP. The results must be effectively communicated to an audience with a wide range of technical backgrounds and interests.

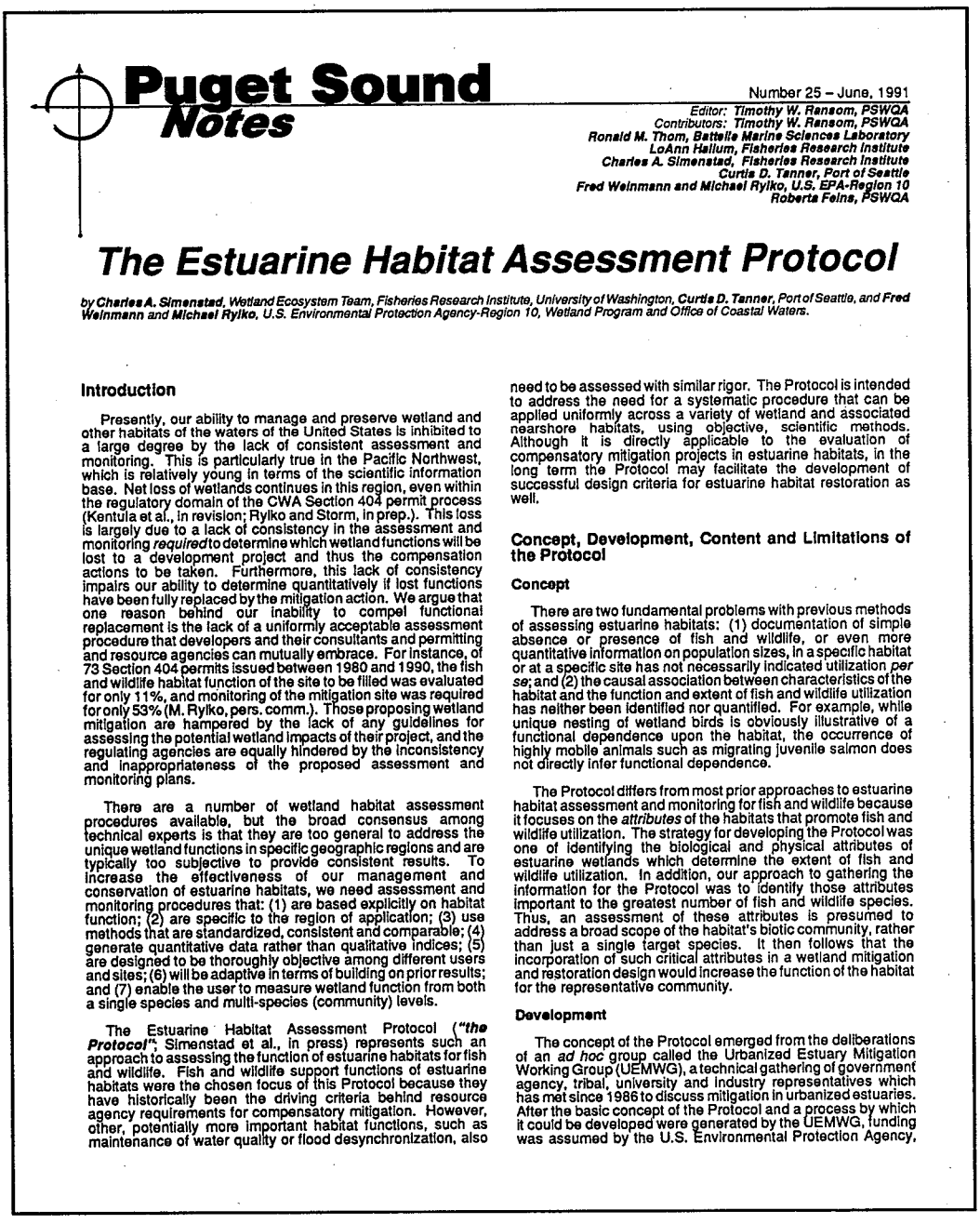
Figure 1-1 and the schematic above show two feedback loops associated with the evaluation of monitoring data. One provides direct feedback of analytical results that are used to modify and refine the monitoring program to increase efficiency (see Section 5.0). The other provides feedback to three basic factors that influence the design, development and refinement of monitoring program objectives: public concerns, modeling, and research. Data analyses must provide information that addresses the needs of program managers, scientists and the public.

Graphical and written summaries should be produced that demonstrate the results of individual components of the monitoring program as well as the relationships between monitoring activities. These summaries should serve as tools to effectively communicate information on the effectiveness of the actions taken under the management plan, and to build public awareness of actions taken by the estuary program. Demonstration materials that summarize program results should be produced for use in newsletters, workshops, poster sessions, and public forums. The results of the monitoring effort and the data analyses should also be made available to the scientific community, and use of the monitoring data should be encouraged. Data analyses should be conducted to test for trends, test and generate new hypotheses, evaluate the uncertainties associated with the data, and to identify the source of these uncertainties. These analyses should serve as a basis for extending existing knowledge of the estuary, making refinements to conceptual and numerical models of the system developed in the characterization phase of the program, and identifying new research. Collectively the analytical results should provide the necessary information for redirecting and refocusing the CCMP.

There are several examples of publications by estuary programs to generate public interest and support, and to disseminate information on monitoring results. In addition to preparing annual technical reports on monitoring efforts, the Puget Sound Estuary Program produces a quarterly newsletter – *Puget Sound Notes* (Figure 7-1). This technical newsletter has a distribution of 2,750 individuals and organizations. It is intended to report on recent program results and to inform interested individuals about events that affect the estuary. The *Chesapeake Bay Barometer* is a one page monthly publication of the Chesapeake Bay Program. The *Barometer* provides a summary of dissolved oxygen concentration and water clarity in the bay during the previous month. It also includes short, nontechnical summaries of topics of general interest (Figure

7-2). Previous topics have included: salinity in the bay, boat pollution, bald eagle populations, and the striped bass fishery. The Santa Monica Bay Restoration Project is producing a series of paired reports on pathogens (Figure 7-3). These reports summarize the results of the long term assessment of inputs of fecal indicators and enteric viruses from storm drains. The results of each phase of the study are summarized in both a *Technical Report* and a *Public Summary*.

Figure 7-1. Sample cover of Puget Sound Notes.



Puget Sound Notes

Number 25 – June, 1991

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The Estuarine Habitat Assessment Protocol

by Charles A. Simenstad, Wetland Ecosystem Team, Fisheries Research Institute, University of Washington, Curtis D. Tanner, Port of Seattle, and Fred Weinmann and Michael Rylko, U.S. Environmental Protection Agency-Region 10, Wetland Program and Office of Coastal Waters.

Introduction

Presently, our ability to manage and preserve wetland and other habitats of the waters of the United States is inhibited to a large degree by the lack of consistent assessment and monitoring. This is particularly true in the Pacific Northwest, which is relatively young in terms of the scientific information base. Net loss of wetlands continues in this region, even within the regulatory domain of the CWA Section 404 permit process (Kentula et al., in revision; Rylko and Storm, in prep.). This loss is largely due to a lack of consistency in the assessment and monitoring *required* to determine which wetland functions will be lost to a development project and thus the compensation actions to be taken. Furthermore, this lack of consistency impairs our ability to determine quantitatively if lost functions have been fully replaced by the mitigation action. We argue that one reason behind our inability to compel functional replacement is the lack of a uniformly acceptable assessment procedure that developers and their consultants and permitting and resource agencies can mutually embrace. For instance, of 73 Section 404 permits issued between 1980 and 1990, the fish and wildlife habitat function of the site to be filled was evaluated for only 11%, and monitoring of the mitigation site was required for only 53% (M. Rylko, pers. comm.). Those proposing wetland mitigation are hampered by the lack of any guidelines for assessing the potential wetland impacts of their project, and the regulating agencies are equally hindered by the inconsistency and inappropriateness of the proposed assessment and monitoring plans.

There are a number of wetland habitat assessment procedures available, but the broad consensus among technical experts is that they are too general to address the unique wetland functions in specific geographic regions and are typically too subjective to provide consistent results. To increase the effectiveness of our management and conservation of estuarine habitats, we need assessment and monitoring procedures that: (1) are based explicitly on habitat function; (2) are specific to the region of application; (3) use methods that are standardized, consistent and comparable; (4) generate quantitative data rather than qualitative indices; (5) are designed to be thoroughly objective among different users and sites; (6) will be adaptive in terms of building on prior results; and (7) enable the user to measure wetland function from both a single species and multi-species (community) levels.

The Estuarine Habitat Assessment Protocol (***"the Protocol"***; Simenstad et al., in press) represents such an approach to assessing the function of estuarine habitats for fish and wildlife. Fish and wildlife support functions of estuarine habitats were the chosen focus of this Protocol because they have historically been the driving criteria behind resource agency requirements for compensatory mitigation. However, other, potentially more important habitat functions, such as maintenance of water quality or flood desynchronization, also need to be assessed with similar rigor. The Protocol is intended to address the need for a systematic procedure that can be applied uniformly across a variety of wetland and associated nearshore habitats, using objective, scientific methods. Although it is directly applicable to the evaluation of compensatory mitigation projects in estuarine habitats, in the long term the Protocol may facilitate the development of successful design criteria for estuarine habitat restoration as well.

Concept, Development, Content and Limitations of the Protocol

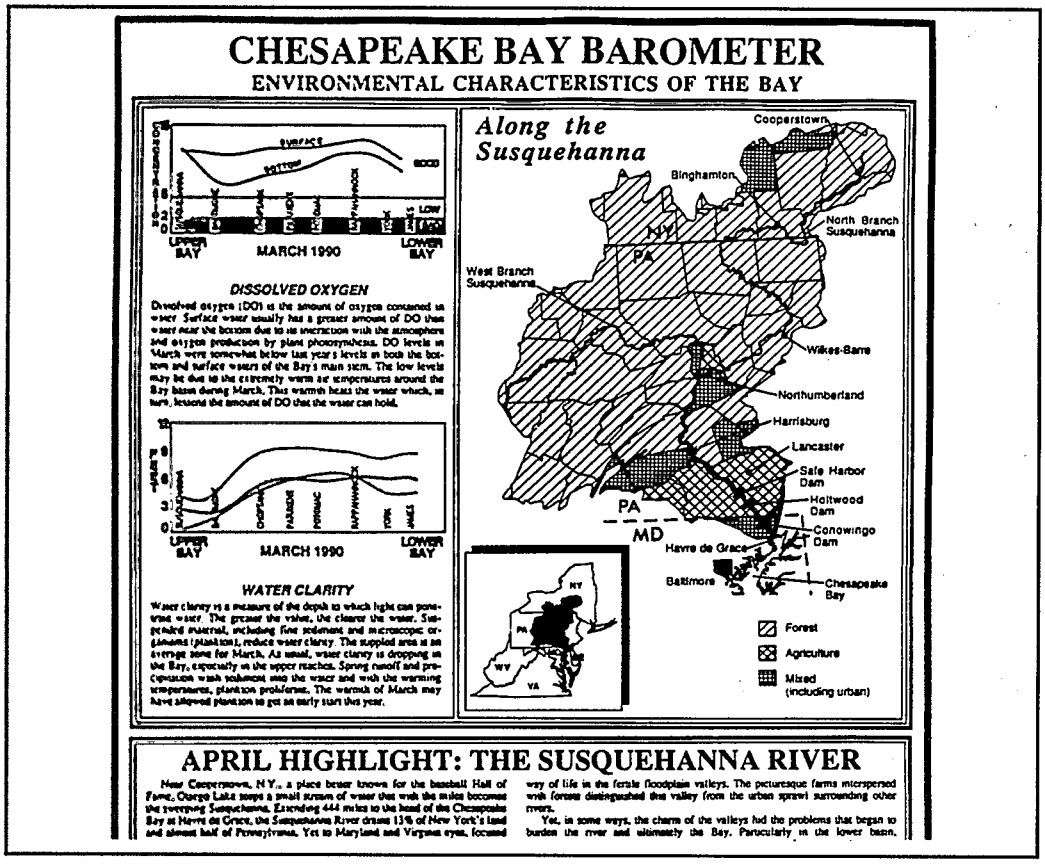
Concept

There are two fundamental problems with previous methods of assessing estuarine habitats: (1) documentation of simple absence or presence of fish and wildlife, or even more quantitative information on population sizes, in a specific habitat or at a specific site has not necessarily indicated utilization *per se*; and (2) the causal association between characteristics of the habitat and the function and extent of fish and wildlife utilization has neither been identified nor quantified. For example, while unique nesting of wetland birds is obviously illustrative of a functional dependence upon the habitat, the occurrence of highly mobile animals such as migrating juvenile salmon does not directly infer functional dependence.

The Protocol differs from most prior approaches to estuarine habitat assessment and monitoring for fish and wildlife because it focuses on the *attributes* of the habitats that promote fish and wildlife utilization. The strategy for developing the Protocol was one of identifying the biological and physical attributes of estuarine wetlands which determine the extent of fish and wildlife utilization. In addition, our approach to gathering the information for the Protocol was to identify those attributes important to the greatest number of fish and wildlife species. Thus, an assessment of these attributes is presumed to address a broad scope of the habitat's biotic community, rather than just a single target species. It then follows that the incorporation of such critical attributes in a wetland mitigation and restoration design would increase the function of the habitat for the representative community.

Development

The concept of the Protocol emerged from the deliberations of an *ad hoc* group called the Urbanized Estuary Mitigation Working Group (UEMWG), a technical gathering of government agency, tribal, university and industry representatives which has met since 1986 to discuss mitigation in urbanized estuaries. After the basic concept of the Protocol and a process by which it could be developed were generated by the UEMWG, funding was assumed by the U.S. Environmental Protection Agency,



CHESAPEAKE BAY BAROMETER

ENVIRONMENTAL CHARACTERISTICS OF THE BAY

MARCH 1990

DISSOLVED OXYGEN

Dissolved oxygen (DO) is the amount of oxygen contained in water. Surface water usually has a greater amount of DO than water near the bottom due to its interaction with the atmosphere and oxygen production by plant photosynthesis. DO levels in March were somewhat below last year's levels in both the bottom and surface waters of the Bay's main stem. The low levels may be due to the extremely warm air temperatures around the Bay basin during March. This warmth heats the water which, in turn, lessens the amount of DO that the water can hold.

MARCH 1990

WATER CLARITY

Water clarity is a measure of the depth to which light can penetrate water. The greater the value, the clearer the water. Suspended material, including fine sediment and microscopic organisms (plankton), reduce water clarity. The supplied area is an average zone for March. As usual, water clarity is dropping in the Bay, especially in the upper reaches. Spring runoff and precipitation wash sediment into the water and with the warming temperatures, plankton proliferate. The warmth of March may have allowed plankton to get an early start this year.

Along the Susquehanna

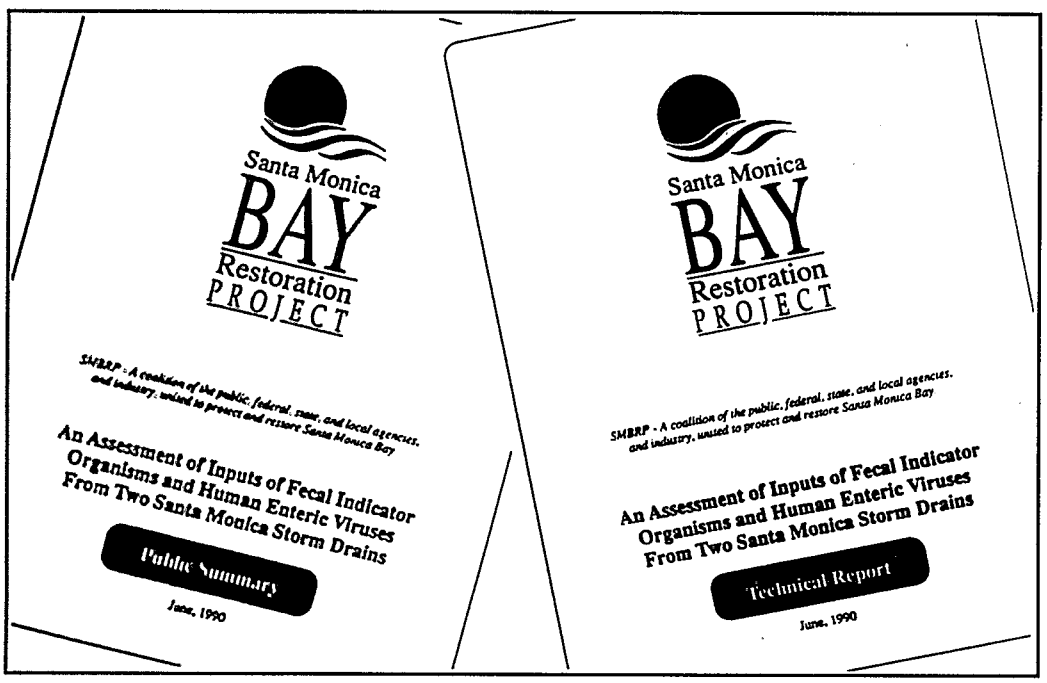
Cooperstown
Binghamton
NY
PA
North Branch Susquehanna
West Branch Susquehanna
Wilkes-Barre
Northumberland
Harrisburg
Lancaster
Safe Harbor Dam
Holtwood Dam
Conowingo Dam
Havre de Grace
Baltimore
Chesapeake Bay
MD
Forest
Agriculture
Mixed (including urban)

APRIL HIGHLIGHT: THE SUSQUEHANNA RIVER

Near Cooperstown, N.Y., a place better known for the baseball Hall of Fame, Otsego Lake seeps a small stream of water that with the miles becomes the sweeping Susquehanna. Extending 444 miles to the head of the Chesapeake Bay at Havre de Grace, the Susquehanna River drains 11% of New York's land and almost half of Pennsylvania. Yet to Maryland and Virginia eyes, focused way of life in the fertile floodplain valleys. The picturesque farms interspersed with forests distinguished this valley from the urban sprawl surrounding other rivers.

Yet, in some ways, the charm of the valleys hid the problems that began to burden the river and ultimately the Bay. Particularly in the lower basin.

Figure 7-2. Sample cover of Chesapeake Bay Barometer.



Santa Monica BAY Restoration PROJECT

SMBRP - A coalition of the public, federal, state, and local agencies, and industry, united to protect and restore Santa Monica Bay

An Assessment of Inputs of Fecal Indicator Organisms and Human Enteric Viruses From Two Santa Monica Storm Drains

Public Summary

June, 1990

Santa Monica BAY Restoration PROJECT

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Technical Report

June, 1990

Figure 7-3. Sample covers of Santa Monica Bay Restoration Project Reports.

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Appendix A – Case Studies

A1.0 The Puget Sound Ambient Monitoring Program Case Study*

A1.1 Purpose and Approach

The Puget Sound Ambient Monitoring Program (PSAMP) is a comprehensive monitoring program which examines environmental variables throughout Puget Sound and the surrounding watersheds. With the designation of Puget Sound as an estuary of national significance in 1988, PSAMP was adopted and submitted as part of the designation package of the National Estuary Program. The development, implementation, management, and coordination of PSAMP can serve as an excellent model for estuarine monitoring programs nationwide.

The sequence of events leading to the implementation of the Puget Sound Ambient Monitoring Program is shown in Figure A1-1. Important features of this time line are that the development of this comprehensive monitoring plan was well underway prior to the selection of Puget Sound for the National Estuary Program, and that the development and implementation of such a comprehensive monitoring program takes substantial time. The PSAMP design was developed between 1986 and 1988, and sampling began in 1989. However, the Puget Sound Water Quality Authority (PSWQA), which played a key role in its development, was formed in 1983 and issued a report in 1984 which recognized the need for estuary-wide monitoring.

Throughout the process of developing PSAMP, National Estuary Program (NEP) objectives were taken into account. PSAMP fulfills the monitoring requirements of the NEP, including:

- identify trends in water quality, natural resources, and uses of the estuary,
- develop the relationship between loads and potential uses,
- identify causes of environmental problems, and
- monitor the effectiveness of actions taken under the Puget Sound Water Quality Management Plan (CCMP).

This case study describes the steps involved in the development of PSAMP, the goals of the program, the monitoring design, and the relationship of PSAMP to the Comprehensive Conservation and Management Plan (CCMP) for Puget Sound. Emphasis is placed on describing the institutional aspects of the monitoring program development process, including the roles of the participating federal, state, local, and tribal governments. The importance of scientific review and input from the public is also emphasized.

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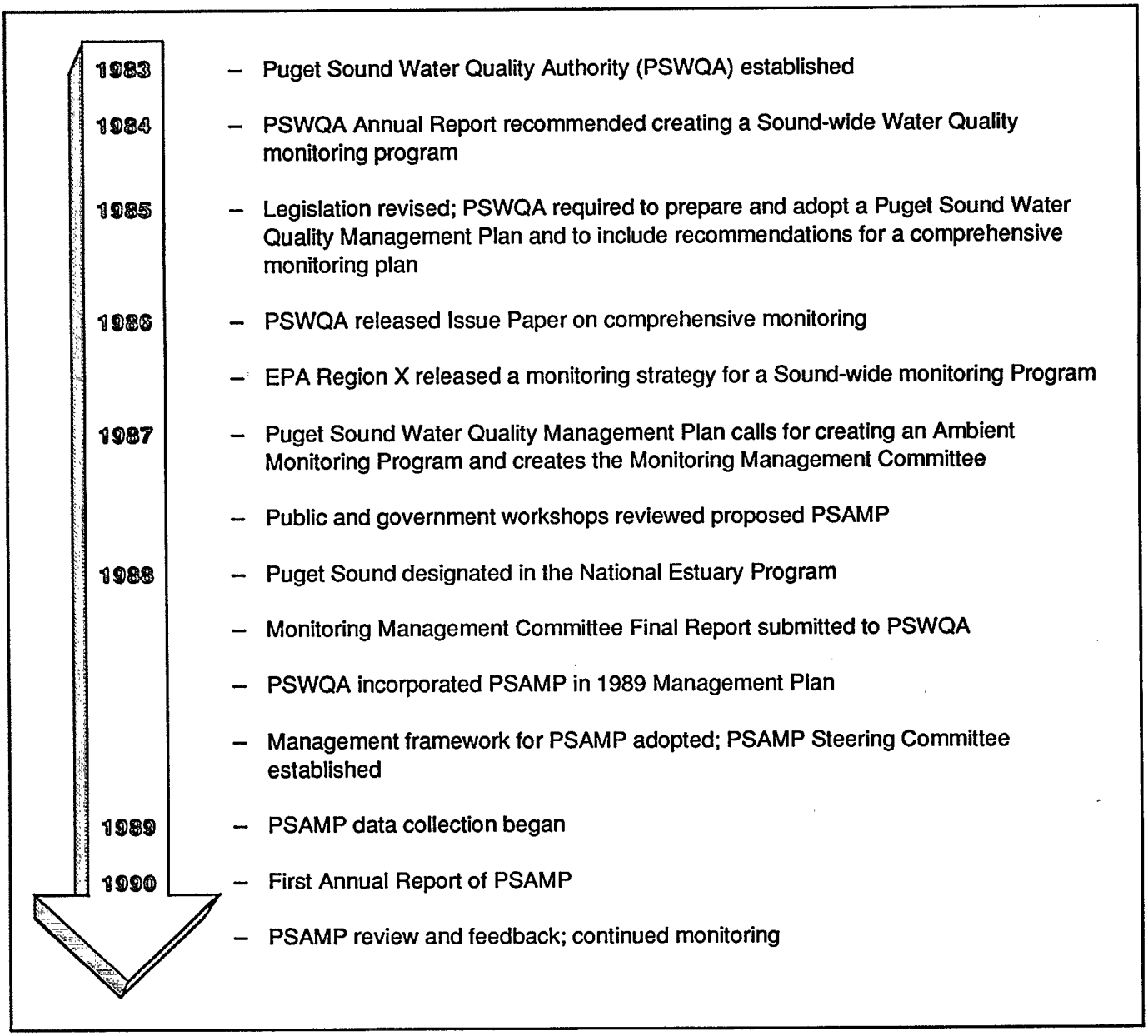
*This case study was adapted from a paper prepared by Andrea E. Copping of the Puget Sound Water Quality Authority and John W. Armstrong of the U.S. Environmental Protection Agency, Region 10, for the January 1991 Puget Sound Research Conference.

Figure A1-1. Events leading to the development and implementation of the Puget Sound Ambient Monitoring Program.



A1.2 Development of PSAMP: Institutional Arrangements

Early in the development of PSAMP, it became clear that the cost and logistics of such a comprehensive effort would require the participation of all key resource agencies and affected parties. Participating local, state and federal agencies would have to make modifications to existing monitoring efforts and agree to share data. It was also

recognized that securing long term funding sources would require strong public support. The key agencies and the milestones in the development and implementation of PSAMP are described below.

The Puget Sound Water Quality Authority

In 1983 the Governor of the state of Washington appointed the 21 member Puget Sound Water Quality Authority (PSWQA) and charged them with identifying pollution-related threats to Puget Sound marine life, evaluating pollution threats to human health, and investigating the need for coordination among agencies responsible for protecting Puget Sound water quality. In their 1984 report, the PSWQA called for "a long-range coordinated plan ...to protect and improve water quality throughout the Sound" (Booth and Powell, 1984). They further recognized the need for an estuary-wide management plan, including increased coordination and enhancement of existing monitoring programs.

In 1985, the Washington state legislature created the PSWQA as a state agency with a seven member board appointed by the Governor. The enabling legislation charged the PSWQA with developing a comprehensive management plan for Puget Sound and its related waterways. The PSWQA was required to prepare and adopt a Puget Sound Water Quality Management Plan by January 1987, to update the plan in 1989 and 1991, and to oversee the implementation of the plan. The legislation required that the plan include consideration of many issues, including "...recommendations for a comprehensive water quality and sediment monitoring program..." PSWQA was also charged with creating a biennial "State of the Sound" report to describe water quality and related resource conditions in Puget Sound. Throughout its history the PSWQA has relied heavily on input from all parties affected by water quality, regulatory frameworks, and the need to manage the resources of Puget Sound.

PSWQA Monitoring Issue Paper

As part of the development of the 1987 Puget Sound Water Quality Management Plan, the PSWQA wrote an issue paper on Comprehensive Monitoring of Puget Sound (PSWQA, 1986). The issue paper reviewed existing Puget Sound monitoring programs, described the process for developing a comprehensive water quality and sediment monitoring program which includes biological resources, and presented alternatives for developing the monitoring program (Table A1-1). The issue paper identified four major components of a comprehensive monitoring program:

TABLE A1-1. ALTERNATIVES FOR IMPROVING PUGET SOUND MONITORING

- Development and adoption of standardized protocols
- A centralized or coordinated data management system
- Improved data interpretation/report preparation and information dissemination
- Adequate laboratory capacity
- Review of existing water column program and changes to collect more pertinent information. Follow up actions could be tied to monitoring results.
- Development of a sediment monitoring program, including ambient sediment monitoring, intensive surveys, discharge monitoring, and dredging/dredged material disposal monitoring
- Improved point and nonpoint source monitoring for municipal treatment plants and industrial facilities, searches for illegal discharges
- Greater number of intensive surveys to identify sources of pollution
- Development of a biological monitoring program
- Improved monitoring of commercial and recreational shellfish beds
- Monitoring by citizen volunteers

Reference: PSWQA 1986.

- ambient monitoring
- discharge monitoring/compliance monitoring
- intensive surveys
- resource monitoring and other programs (e.g., monitoring paralytic shellfish poisoning, changes in shoreline and habitat, weather, demographics, and socioeconomic information.)

The PSWQA included findings of the 1986 issue paper, and comments received on the issue paper, in the 1987 Puget Sound Water Quality Management Plan (PSWQA, 1987).

EPA Region 10 Monitoring Guidance Report

In 1986, the Office of Puget Sound, EPA Region 10, directed their consultant to develop a monitoring strategy for a Sound-wide monitoring program. Through a process involving consultation with agencies concerned with Puget Sound management, the consultant developed a monitoring design which included goals and objectives, sampling design, operation of the program, and methods for sampling, analysis, and reporting of data. The monitoring design was released in draft form in 1986 (Tetra Tech, 1986). At that point EPA Region 10 and the PSWQA recognized the need for a process which would further involve the many agencies, organizations, and individuals with an interest in Puget Sound monitoring.

Monitoring Management Committee

The 1987 plan required the PSWQA to appoint a Monitoring Management Committee (MMC) (PSWQA, 1987). The MMC is chaired by the PSWQA and consists of representatives of state, federal, local, and tribal governments, business, industry, shellfish growers, university scientists, Canadian agencies, environmental groups, and the public. The plan directed the MMC to develop a comprehensive ambient monitoring program, and to address 16 specific issues related to the program (Table A1-2). Staff support was provided to the MMC to carry out coordination and data management tasks.

The MMC was appointed in September 1986. Like all PSWQA meetings, MMC meetings are open to the public. The committee reaches all decisions by consensus.

The committee began its work in late 1986 by defining the goals and objectives of the monitoring program and then proceeded to review and modify the consultant monitoring strategy (Tetra Tech, 1986). The MMC established technical subcommittees to study the monitoring design and to make recommendations on program design.

From late 1986 to early 1988 the MMC refined the monitoring program design which included sampling and analysis recommendations, a data management system design, quality assurance guidelines, reporting requirements, cost estimates, and funding alternatives. The committee released a draft program design in May 1987 for public and scientific peer review. Workshops were held for the public, local government, and tribal staff. Comments received from the PSEP technical advisory committee (TAC), scientists, agency staff, and other affected parties were considered by the committee and a final report entitled "Puget Sound Ambient Monitoring Program - Detailed Design Considerations" was adopted by the PSWQA and published in April 1988

TABLE A1-2. MMC ACTIVITIES REQUIRED BY PSWQA IN DEVELOPING PSAMP

- Define the goals and objectives of the monitoring program.
- Define how the monitoring data will be used to assist in management of Puget Sound resources by responsible agencies.
- Review and provide for the specific monitoring needs of programs in the Puget Sound Water Quality Management Plan.
- Propose a monitoring design based to the extent applicable on the November 1986 consultant report to EPA on Puget Sound monitoring and review a similar report on freshwater (watershed) monitoring.
- Analyze the data needed to meet the objectives of the monitoring program.
- Analyze and evaluate existing programs and their relation to the objectives of a monitoring program.
- Identify comprehensive monitoring needs not being met by existing programs.
- Identify mechanisms to obtain needed information either through redirection of existing programs or initiating of new programs.
- Estimate annual costs associated with existing monitoring in Puget Sound and costs of additional needed monitoring.
- Recommend a data management system that meets agency needs.
- Define appropriate mechanisms to fund the monitoring program including allocation of existing funds and new funding.
- Define the appropriate roles for agencies in implementing the monitoring program.
- Define how the Puget Sound Atlas (Evans-Hamilton and D.R. Systems 1987) would be updated, managed, and integrated with ongoing activities.
- Recommend a structure for managing the monitoring program, including mechanisms for making needed refinements and adjustments to ensure program objectives are met in a cost-effective manner.
- Identify opportunities for citizens' monitoring of Puget Sound.
- Form ad hoc technical working groups to assist in developing the monitoring program.

Reference: PSWQA, 1987.

(PSWQA, 1988a). The report included PSAMP goals and objectives, and sampling and analysis strategies for monitoring sediment quality, marine and fresh water quality, fish, shellfish, birds, marine mammals, river mouths, nearshore habitat, and additional data. The PSAMP design was incorporated by reference into the 1989 Puget Sound Water Quality Management Plan (PSWQA, 1988b).

A1.3 The Puget Sound Ambient Monitoring Program

The MMC developed five goals for PSAMP:

1) Characterize and interpret spatial and temporal patterns of conditions of Puget Sound in relation to its natural resources and for humans, and recognize contamination.

2) Take measurements to support specific program elements identified in the Puget Sound Water Quality Management Plan.

3) Measure the success of programs implemented under the Puget Sound Water Quality Management Plan (as they relate to the overall ambient monitoring goal and the program goals of the plan).

4) Provide a permanent record of significant natural and human-caused changes in key environmental indicators in Puget Sound over time.

5) Support research activities through the availability of consistent, scientifically valid data.

In order to achieve these goals, the monitoring program characterizes and interprets spatial and temporal patterns for the following:

- factors that endanger human health
- biological populations and communities
- factors affecting biological populations
- presence of pollutants in the Sound
- entry of pollutants into the Sound from rivers and streams
- estuarine and wetland habitats
- results of water quality degradation, such as shellfish bed closures
- improvements in water quality such as the reopening of shellfish beds
- factors that affect aesthetic conditions.

The monitoring program identifies areas where:

- resources are damaged or contaminated
- resources or conditions are changing over time
- intermittent or periodic degradations are occurring.

The Scope of PSAMP

The design of PSAMP calls for monitoring of sediments, water quality in marine and fresh waters, fish, shellfish, birds, marine mammals, and nearshore habitat. The focus of the program is Puget Sound-wide, which includes Hood Canal, portions of the Straits of Juan du Fuca and Georgia to the Canadian border, and the surrounding watersheds. The sediment quality sampling stations, for example, are shown in Figure A1-2. Sampling occurs year-round. Station locations, the number of stations or surveys, and the timing of sampling varies with each type of measurement. Three types of stations are sampled in many tasks of the program:

- fixed stations that are sampled each year,
- rotating stations that are sampled on a 3 year cycle; these stations can provide extended geographic coverage, and
- a small number of floating stations that are located at the discretion of the implementing agency; these stations can provide additional geographic coverage, or allow investigation of a suspected water or sediment quality problem.

Table A1-3 summarizes the design for full PSAMP implementation, as well as the monitoring data that are being collected during 1989-90.

Citizens' monitoring is an integral part of PSAMP. The advantages of using citizens to collect data are twofold: samples and observations can be collected in a cost-effective manner, particularly at remote locations where travel by agency staff is costly; and as a tool for increased public involvement, education, and stewardship. Using state funds, PSAMP contracts with citizens' groups to provide volunteers to collect information and samples under the guidance of implementing agency staff (funds are used for administrative purposes and to reimburse expenses). Parameters for collection by citizen monitors are carefully chosen to minimize quality assurance problems; for example, citizens are used extensively to dig shellfish for tissue analysis, to catch marine fish and salmon for tissue analysis, and to groundtruth remote sensing data.

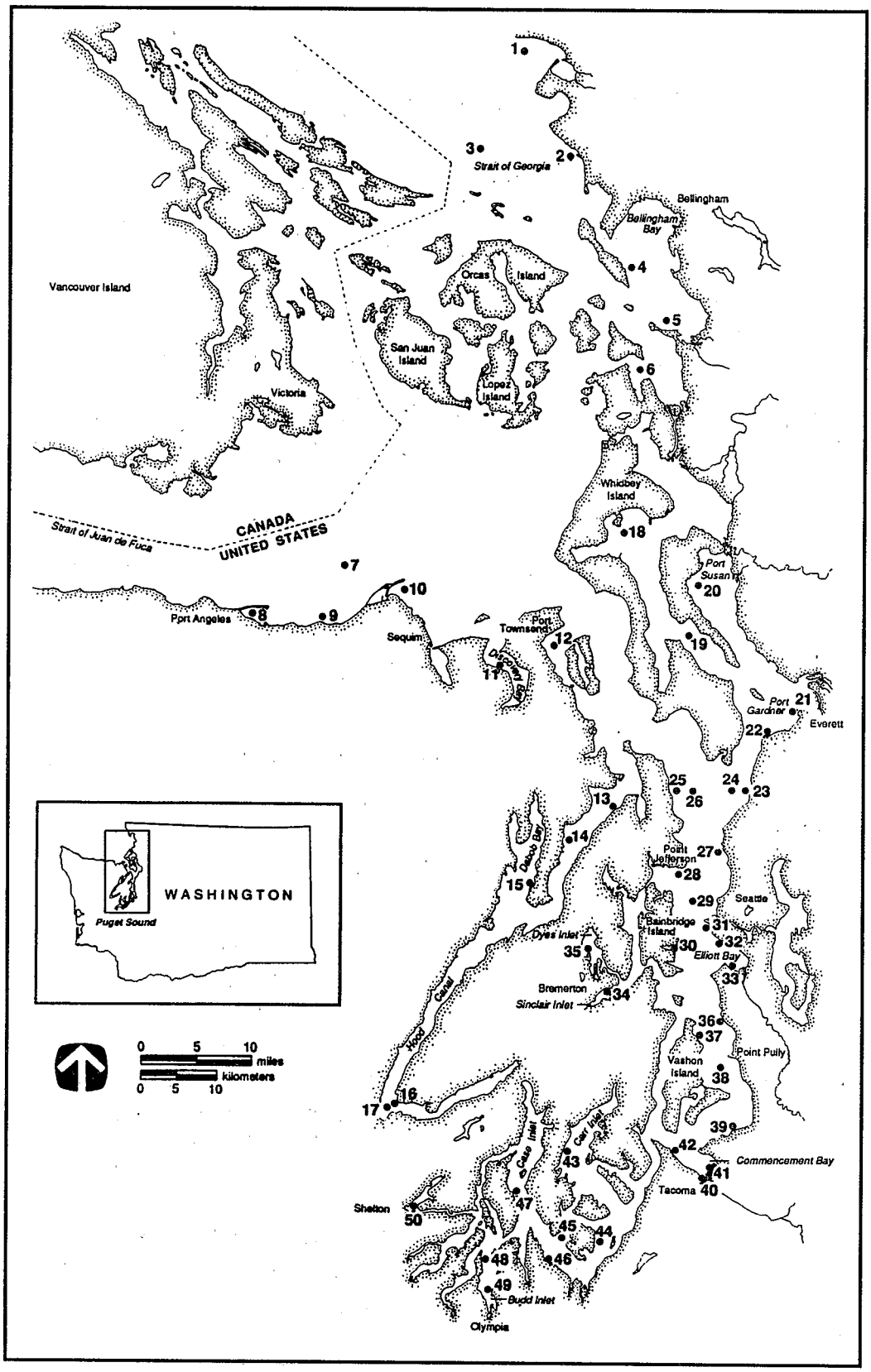


Figure A1-2. Area included in Puget Sound Ambient Monitoring Program.

TABLE A1-3. PSAMP DESIGN AND 1989-90 ACTIVITIES

Task	Task Subcomponents	Proposed No. of Stations/Surveys	Actual 1988-89 Stations/Surveys	Proposed Sampling Frequency	Actual Sampling Frequency
Sediment quality	Sediment chemistry Bioassays Benthic invertebrates	75 throughout Puget Sound	50 throughout Puget Sound	Annually-spring	March-April (1989, 1990)
Marine water column	Long-term trends	10-12 throughout Puget Sound	24 throughout Puget Sound	Monthly	Monthly
	Known water quality problems	5-10 in selected bays			Seasonally
	Algal growth	5-10 in selected bays			Summer/winter solstices
Fish	Tissue chemistry (bottomfish)	21 stations	10 stations	Annually-early summer	May 1989
	Liver histopathology (bottomfish)	21 stations	10 stations	Annually-early summer	May 1989
	Tissue chemistry (cod, rockfish, salmon)	5-10 stations	4 stations	Annually, depending on species	September/ February/ April 1989-1990
Shellfish	Abundance	35 beaches	10 beaches	Annually	May 1990
	Bacterial contamination	35 beaches	10 beaches	Quarterly	Quarterly
	Tissue chemistry	35 beaches	4 beaches	Annually	April 1990
	Paralytic shellfish poisoning	35 beaches	16 beaches	Monthly	Monthly
Birds		Throughout Puget Sound	No activity	Monthly, annually	
Marine mammals		Throughout Puget Sound	No activity	Monthly, annually	
Nearshore habitat		One-third of Puget Sound	No activity	Annually	
Freshwater		75 throughout watershed	75 throughout watersheds (limited parameters)	Monthly	Monthly

Reference: PSWQA 1990a.

The NEP and PSAMP

With the formal designation of Puget Sound as an estuary of National Significance in March 1988, the 1991 Puget Sound Water Quality Management Plan was adopted as the Comprehensive Conservation and Management Plan, and PSAMP formally became a part of Puget Sound Estuary Program.

PSAMP examines data from selected other monitoring programs and studies underway in the Puget Sound basin in order to better interpret PSAMP data, and to meet the needs of the NEP which states that a CCMP "shall survey and utilize existing reports, data, and studies relating to the estuary that have been developed by or made available to federal, interstate, state, and local agencies." Monitoring programs and studies that evaluate environmental conditions and potential sources in the Puget Sound region that are of interest to PSAMP include:

- nonpoint source control watershed monitoring
- Puget Sound Dredge Disposal Analysis (PSDDA)
- PSEP Urban Bay Studies (UBATs)
- NPDES compliance monitoring
- Department of Ecology intensive surveys (tracing sources of contamination)
- NOAA National Status and Trends Program
- research studies, historical information, and other studies.
- Timber/Fish/Wildlife
- climate/weather data
- demographic and socioeconomic conditions
- decision record-keeping.

A1.4 Implementation of PSAMP and Cost

Assuring PSAMP Implementation

In order to ensure that PSAMP would be implemented as planned, the PSWQA negotiated memoranda of agreement (MOA) with each of the implementing agencies. These agreements detailed the specific responsibilities of each implementing agency and the PSWQA in relation to PSAMP. The MOAs specify that each implementing agency must carry out its responsibilities under the PSAMP design, must maintain PSAMP funding levels within the agency unless the Governor or the state legislature requires across-the-board agency funding cuts, and must refer changes in PSAMP to the PSAMP Steering Committee for approval. Through the MOAs, the PSWQA

agrees to provide staff support to the PSAMP committees and to work with the implementing agencies to maintain long-term funding for PSAMP.

Under the 1985 legislation creating the PSWQA, the agency was due to terminate in 1991. In order to ensure the future of PSAMP, during fall 1989 the PSAMP Steering Committee developed a recommendation for future management structure for PSAMP which was not markedly different from the existing one. The PSAMP Steering Committee preferred that the coordination and dispute resolution functions remain with the PSWQA. The committee's recommendation was incorporated into the PSWQA enabling legislation in 1990, which reauthorized the PSWQA. The new section in the legislation which deals with PSAMP requires the implementing agencies to participate in the program, requires the PSWQA to ensure implementation of PSAMP, and allows the PSWQA to establish an interagency committee to coordinate PSAMP.

Initial PSAMP Assignments

Agencies represented on the MMC were asked to express their interest in playing a lead role in specific tasks of PSAMP. "Implementing agencies" were chosen for each PSAMP task based on these expressions of interest. It came as no surprise that the agencies that expressed interest in becoming implementing agencies were those with regulatory and/or management responsibilities as well as expertise in the specific areas; initially all implementing agencies are state agencies (Table A1-4), although this could change in the future. Each implementing agency was responsible for preparing a detailed implementation plan, managing their specific PSAMP task, quality assurance and quality control, data management and reporting, and transfer of PSAMP data to the central database, housed at the PSWQA. The MMC felt strongly that the PSWQA should continue to play the lead role in coordinating PSAMP and providing data management functions, as the Authority has both the expertise and staff available to carry out these functions.

Cost

A technical subcommittee to the MMC (Costing Subcommittee), PSWQA staff and a consultant retained by PSWQA worked to develop a cost estimate for PSAMP. In 1987 dollars, full implementation of PSAMP was calculated to cost approximately $3.2 million a year, with startup costs of $250,000 to $350,000 over the first two years. Early in 1988 it became apparent that state funds would not be available for full implementation of the program. Through a priority-setting process involving the

TABLE A1-4. PSAMP TASK ASSIGNMENTS BY AGENCY

Monitoring Task	Implementing Agency
Sediment Quality	Washington Department of Ecology
Marine Water Column	Washington Department of Ecology
Fish	Washington Department of Fisheries
Shellfish	Washington Department of Health
Birds	Washington Department of Wildlife
Marine Mammals	Washington Department of Wildlife
Nearshore Habitat	Washington Department of Natural Resources
Freshwater	Washington Department of Ecology

Reference: PSWQA 1990b

MMC and other scientists, a plan for phased implementation of the program was devised. This process established the order in which PSAMP tasks would be funded when implementation funding became available. The MMC established sediment quality, fish tissue, and shellfish tissue monitoring as top-ranked priorities for funding. The MMC also recognized that agency staff could not manage to fully implement PSAMP in a single year, even if funds were to become available.

The development phase of PSAMP, including establishment of the data management system, has been funded largely by PSEP funds administered by EPA Region 10 and made available to the PSWQA by cooperative agreement. State, federal, local, and tribal agencies, as well as private sector funds have contributed individuals' time serving on monitoring committees and subcommittees. The development costs of PSAMP were approximately $200,000 from 1987-1989. Additional development costs for data management are still being incurred.

Funding

Long-term stable funds for monitoring programs are notoriously difficult to secure. Funding for four types of activities were needed to assure the success of PSAMP: contractor funds for the development of the draft monitoring strategy and several workshops; staff time for program development; startup costs; and ongoing implementation. As previously noted, EPA Region 10 provided PSEP funds for PSWQA staff to coordinate the development of PSAMP and the data management system. PSEP resources continue to fund PSWQA staff for initial implementation and further database development through FY91. Start up costs for implementing PSAMP have been itemized together with long-term implementation costs to clarify what is needed to properly initiate such a program.

To date, approximately 30% of PSAMP has been funded, including central coordination and data management tasks. Other than PSEP funds available through EPA, all PSAMP funding has come from the state of Washington. Like all Puget Sound Water Quality Management Plan funds, PSAMP funds are given directly to the implementing agency; the PSWQA submits a funding request for the entire plan, with assistance from the other agencies, and acts as an advocate for the plan to the Governor and the state legislature.

During 1989-90, PSAMP received approximately $900,000 of state general fund money each year (PSWQA 1990a). The Washington state Department of Ecology receives $500,000 a year for sediment sampling and analysis. $200,000 goes to the Washington state Department of Fisheries for measuring toxics in bottomfish and recreational fish tissue and for bottomfish health assessments. The Washington state Department of Health uses $160,000 for sampling and analysis of shellfish tissue for bacterial and/or chemical contamination. The PSWQA receives $40,000 a year for coordination and data management. $50,000 in citizens' monitoring activities are funded by a special fund for public involvement and education (PIE Fund) from the state Centennial Clean Water Fund (tax on tobacco products). In addition to securing new funds, the Washington state Department of Ecology has modified existing marine and fresh water monitoring programs to meet PSAMP goals, saving PSAMP $245,000 a year.

Maintaining current PSAMP funding and securing additional funds is a major focus of the PSAMP Steering Committee and the PSWQA. The MOAs are intended to protect PSAMP funds within the implementing agencies, all of which have numerous conflicting priorities for funds. The monitoring committees and the PSWQA are relying

heavily on legislative advocacy, attention from the media, and garnering of public support through the dissemination of PSAMP results to aid in securing PSAMP funds.

Management of PSAMP

The management structure for PSAMP consists of the PSAMP Steering Committee, the MMC and subcommittees, and the PSWQA board. PSWQA staff provide technical and administrative support to the committees, and chair the MMC and PSAMP Steering Committee.

As PSAMP moved from the development to the implementation stage, it became apparent that the MMC was too large and unwieldy a group to coordinate the program and make day to day decisions. In addition, some state agencies were uncomfortable with having representatives from the private sector make decisions involving the use of public funds. To solve these problems, a second monitoring committee was formed, the PSAMP Steering Committee.

The PSAMP Steering Committee is chaired by the PSWQA and is made up of one representative from the five implementing agencies, the PSWQA, EPA Region 10, local government, and tribes. Additional members can be added in future if necessary. The committee is responsible for the overall coordination and management of PSAMP. The PSAMP Steering Committee meets once a month and reaches decisions by consensus.

Should the PSAMP Steering Committee be unable to reach consensus on technical or other program matters, or should any member be dissatisfied with decisions made by the committee, the matter can be referred to the PSWQA board for resolution. To date, there have been no such instances.

The MMC maintains the same representation that it had during the development phase, and, with its subcommittees, acts as the advisor to the PSAMP Steering Committee. The MMC meets twice a year or as needed. Major changes in PSAMP design, changes in assignment of an implementing agency, setting funding priorities, and other technical issues must be referred to the MMC by the PSAMP Steering Committee.

Although the PSWQA has played the central role in facilitating the development and initial implementation of PSAMP, the PSWQA is committed to maintaining PSAMP as an interagency effort which benefits the public, as well as many agencies and organizations besides the PSEP co-managers and implementing agencies.

Review of PSAMP

The PSAMP Steering Committee and the MMC recognize that the long-term success of PSAMP rests on making the program effective in answering needs of the resource management agencies, and informing the public of the state of the Sound, within the available budget. An annual review of PSAMP is built into the process by the participants in order to keep the program focussed on top priority monitoring needs and to ensure the efficient use of funds. The first annual review will begin in late 1990. In addition, PSAMP will undergo periodic independent review; the first review is set for 1994, with subsequent reviews every 3 years.

Data Management, Quality Assurance and Reporting

Data management and quality assurance/quality control (QA/QC) requirements for PSAMP were designed as integral parts of the program. The data management system consists of a central database housed at the PSWQA, and agency databases at each of the implementing agencies. The databases are microcomputer (desktop computer) based, and exchange data electronically using a set of standardized data transfer formats developed by PSWQA staff in cooperation with technical subcommittees to the MMC. The central database and several of the agency databases are on dBase IV and have been customized by PSWQA staff.

PSAMP is also responsible for developing a geographic information system (GIS) on Puget Sound. Resource maps from the Puget Sound Environmental Atlas (Evans-Hamilton and D.R. Systems, 1987) have recently been converted from graphics files to Arc-Info (GIS program) by the USGS, under an agreement with the PSWQA. PSWQA staff are presently working with the Washington State Department of Natural Resources to update the Atlas on GIS and will complete the initial Puget Sound GIS by 1993.

Each implementing agency is responsible for QA/QC of sample collection, analysis, and data management for their tasks of PSAMP. Limited QA/QC of data are performed at the PSWQA before data are accepted into the central database. QA/QC guidelines were established by the MMC and are detailed in each agency's implementation plan.

Each implementing agency is required to prepare an annual technical report detailing their PSAMP monitoring over the previous year. Using the agency technical reports and other program information, each year PSWQA staff prepare an integrated PSAMP

report for public release. The first of these reports was released in May 1990 (PSWQA, 1990a). PSAMP reports and quality assured data are available through the PSWQA.

A1.5 Summary and Recommendations

This case study describes the process by which agencies, the private sector, scientists, and the public interest groups interacted to design and implement PSAMP. It also identifies some of the ingredients that have made it a successful process. Additionally, there are several opportunities for other estuary programs to improve upon this process.

Keys to Success

The success of PSAMP design process is attributable to the agencies and individuals who saw the need for a comprehensive monitoring program and who made the commitment to make the process work. The PSWQA played an essential role in coordinating and managing the process. One key to the PSWQA's past and ongoing successes has been the agency's comprehensive approach to complex issues by simultaneously addressing the scientific, resource management, political, and public concerns. PSWQA staff have spent considerable effort trying to balance these often conflicting pressures. PSWQA has been able to maintain an objective role in managing and coordinating PSAMP without competing with the individual agencies; PSWQA does not carry out any sampling operations.

The EPA's Office of Puget Sound was very supportive of the efforts to develop PSAMP. Not only did EPA provide funds for the development of the program, but key staff were very much involved in the process and fully supportive of the strong role taken by the PSWQA in coordinating the program. EPA Region 10 has supported the development of a number of other products and studies which have laid the groundwork for PSAMP, including the Puget Sound Protocols and Guidelines; the Puget Sound Environmental Atlas; reconnaissance studies for sediment and tissue contamination; seafood risk assessment study; and the Puget Sound chemicals of concern matrix.

The interest among agencies, legislators, and the public in Puget Sound water quality during the early 1980s helped set the stage for creating the PSWQA and emphasizing water quality issues and programs, such as PSAMP. Prior to the establishment of the NEP, EPA Region 10 and the Washington state Department of Ecology established the Puget Sound Action Program. These agencies were then joined by the PSWQA and formed the PSEP.

The design and initial implementation of PSAMP, including the data management system, is successful both as a technical product and for the consensus process from which it came. The involvement of representatives from both the public and private sectors, a targeted outreach effort, and the process of peer review have all contributed to the success with which the program has been received by the implementing agencies. The data management system and data transfer formats has been very well received by state and local agencies. PSWQA staff have had numerous requests for information, and interest in PSAMP have been communicated from several of the established and emerging estuary programs around the country as well as from other state programs, other regions, and from Canada. Staff are frequently asked to present the program design, institutional structure, data management criteria, and uses of PSAMP data at seminars and conferences in the Puget Sound region and across the country. In addition, PSAMP was able to release its first annual report one year after initial sample collection.

The GIS recommendation was initially prepared to serve many purposes. Funding constraints now and in the future have necessitated that the effort be reduced to fit the immediate needs of updating of the Puget Sound Atlas and providing some geographic data management for PSAMP. The flexibility of the recommendation and the broad-based consensus through which it was built have allowed for this redirection of effort without a loss of time or momentum.

The redirection of existing state agency funds and the procurement of new state money for PSAMP were also aided by the long consensus process that was followed. Although sufficient state funds for full implementation of PSAMP are not currently available, almost $1 million a year is currently committed to PSAMP, and additional resources can be expected to follow in the future.

Opportunities for increased productivity and cost containment have been seized on several occasions during this project. For example, a combination of PSEP and state funds were used to develop the fish task implementation plan and to sample bottomfish during 1989. Similarly, a mix of funds were used to design and develop the PSP database. These opportunities have allowed those agencies to proceed with PSAMP implementation without delay when state funds became available in July 1989. Similarly, the planning carried out by Fisheries and the responsiveness of Fisheries and Authority staff allowed the cost-effective "piggybacking" of bottomfish sampling with other state sampling programs in May 1989.

The transition to a new management structure for PSAMP during the implementation phase, including the establishment of the PSAMP Steering Committee and the reallo-

cation of management responsibilities between the PSAMP Steering Committee and the MMC was accomplished smoothly. The agencies involved displayed a sense of stewardship for the program and have continued to actively participate. The success of the management structure is most clearly illustrated in the incorporation of the PSAMP Steering Committee's recommendation for managing PSAMP into law during the 1990 state legislative session.

All of the implementing agencies are large complex agencies with, in some cases, several divisions involved in the implementation. Despite the complexity of the issues involved in long-term implementation and coordination of PSAMP, three of the five implementing agencies have signed memoranda of agreement with the Authority.

The establishment of a successful citizens' monitoring program benefits both PSAMP through cost-effective collection of data, and educational and public involvement needs. Encouraging an educated and involved public is key to providing support and commitment to the long-term funding of PSAMP.

Opportunities to Improve Future Projects

Throughout the many successes of PSAMP design and initial implementation, there were setbacks, minor difficulties, and areas for future improvement. The majority of these problems could be alleviated in other programs with a combination of lessons learned from this program and the addition of more staff time.

The time to negotiate the memoranda of agreement between each implementing agency and the Authority was seriously underestimated. The size and complexity of several of the agencies involved in the program was not taken into account.

Questions have arisen during the design and initial implementation phases of PSAMP as to whether it would have been preferable to design a more limited and affordable ambient monitoring program, rather than the necessary and sufficient program designed to meet the goals set out in the MMC final report (PSWQA, 1988a). A smaller program would have been designed somewhat differently, and would not have raised the expectations of all those eagerly awaiting PSAMP data. The design of PSAMP as a thoroughly adequate program for characterizing Puget Sound and measuring the success of source control and remedial action programs will act as the blueprint with which to direct funds as they become available. The danger in designing a less comprehensive program is a level of complacency among funding sources and managers who feel that we are collecting adequate information on Puget Sound. With benefit of hindsight, we would probably design much the same program again.

Similarly, the amount of effort that went into the design and implementation of the central database has been questioned as little use has been made of the analytical capabilities of the system to date. The modular design of the central database has allowed portions of it to be customized for use by the individual implementing agencies. Without the PSWQA staff effort in developing the central database, it is unlikely that the implementing agencies would have functional databases in time to manage their early years of PSAMP data. Additionally, as more data are collected under PSAMP, the central database will become the site of integrated analyses among the PSAMP tasks.

Trade offs between efficiency and the need to build broad-based consensus on all parts of PSAMP were seen throughout the design and initial implementation process. For example, additional portions of the central database could have been completed by the end of the development phase if PSWQA staff had begun programming and file design work earlier. However, an earlier start on database development would not have allowed as complete review and participation by the implementing agencies, other participants in PSAMP, and the interested public.

It has proved to be very important to staff the design and implementation of PSAMP with a full-time manager/coordinator and a systems analyst. The task of keeping all the implementing agencies and other participants informed of progress, resolving disputes and misunderstandings, carrying out technical and administrative duties, coordinating with other closely related monitoring and research programs, and publicizing the existence and results of the program was overwhelming for PSWQA monitoring staff. In order to really cover all these bases, additional junior level staff was needed. In future projects, creative use should be made of interns and entry level professional staff, as well as additional funding sought for central coordination and data management.

At times technical questions brought to the MMC and PSAMP Steering Committee could not be adequately resolved due to the lack of technical expertise on the committees. Technical subcommittees were formed and proved useful for many questions; however, inadequate PSWQA staff time did not allow for sufficient meetings and contacts to resolve every need. In addition, there was frequently insufficient time and attention given to the program by committee members. In some instances additional PSWQA staff time could have improved the situation but in general there needs to be a strong devotion to the program by some of the participants, especially the implementing agencies. It is also important to recognize that there is only a finite pool of Puget Sound scientists and managers available to participate on a regular or frequent basis in such programs; a similar-sized pool of experts may prove limiting in many areas.

The major activity which was given short shrift during the initial implementation of PSAMP, and which future projects should strive to improve upon was the publicizing of monitoring data, results, expectations, and interpretations. Even with a very limited amount of new monitoring data there are ample historical Puget Sound data residing in the state and federal agency files which could be resurrected, compared to recently collected information, and displayed to managers and the public. Publicly releasing results and interpretations of scientific findings helps to raise the level of awareness and support for a program like PSAMP.

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A2.0 Chesapeake Bay Monitoring Program: Detection of Trends in Estuaries

A2.1 Purpose and Approach

This case study demonstrates the use of statistical methods to evaluate the ability of ongoing monitoring efforts to detect long term trends in the estuary. As described in Section 5.0, this type of evaluation can be used to determine if the overall goals of the monitoring program are being met and if the existing program should be modified. Water quality data collected as part of the Chesapeake Bay Monitoring Program were obtained to demonstrate the analytical approach. However, this case study is not intended as a review of the ongoing monitoring efforts in the Chesapeake Bay. The data that were used represent a small subset of the data that have been collected as part of a comprehensive sampling effort. The ongoing monitoring program also has many objectives that are not addressed in this example analysis.

Background information on the Chesapeake Bay Program is included, and the development and implementation of the nutrient reduction strategy that is a cornerstone of the bay-wide management plan is described. A description of the water quality data that were obtained from the Chesapeake Bay Program is also included. The focus of the case study, however, is on describing the use of statistical analyses to evaluate monitoring effectiveness and the application of the analytical results. An understanding of statistical power analysis is necessary to fully understand the procedures described. Familiarity with the information provided in section 5.0 is therefore recommended.

A2.2 Chesapeake Bay Program

The Chesapeake Bay Program, first authorized by Congress in 1977, has developed a "long-term process of collecting critical environmental data throughout a 66,500 square mile ecosystem" (Chesapeake Bay Program Monitoring Subcommittee, 1989). Due to the size of the bay, a cooperative effort between numerous state and federal agencies was required to build the current program. Efforts during 1976 through 1983 were spent developing a program and communication structure.

Early characterization efforts indicated that:

- The bay was over-enriched with nutrients
- Toxic "hot spots" exist
- Submerged aquatic vegetation habitats had been lost
- Lowered dissolved oxygen levels were causing serious water quality problems and the loss of valuable habitats

In particular, increases in the extent of oxygen deficient waters and nuisance algae and declines in water clarity, submerged aquatic vegetation, finfish, and oysters (Flemer *et al.*, 1983) were observed. Eutrophication was suspected to be the cause of many of the problems. Though toxics were a major concern, there was much more uncertainty about the cause-effect relationships (Magnien and Haire, 1989).

In 1983, the first Chesapeake Bay Agreement was signed by Maryland, Virginia, Pennsylvania, the District of Columbia, and EPA. The agreement signified a commitment to establish a structure to oversee cooperative and comprehensive measures needed to restore the bay.

In 1984, the first basin-wide monitoring network was established along with a wide range of new initiatives and legislation passed in all three States and the District of Columbia. Previous monitoring information was insufficient to define the changes in water quality between the 1950's and 1970's. Therefore a long term monitoring program was designed to improve the understanding of the bay dynamics associated with pollution reduction, to characterize the current status of water quality problems, and to determine the long-term effects of management actions (e.g., phosphate detergent ban, improved sewage treatment, nutrient reduction strategy, and best management practices).

1987 Chesapeake Bay Agreement

The Chesapeake Bay Program has evolved over a number of years. The 1987 Chesapeake Bay Agreement was especially important because it set forth a water quality goal to "reduce and control point and nonpoint sources of pollution to attain the water quality condition necessary to support the living resources of the Bay." As a result of this commitment by the Governors of Virginia, Maryland, and Pennsylvania, the mayor of the District of Columbia, the Chesapeake Bay Commission, and the EPA Administrator, bay-wide strategies for the control and reduction of nutrients, toxics, and conventional pollutants were to be developed.

Nutrient Reduction Strategy

The 1987 Chesapeake Bay Agreement established a commitment to develop, adopt, and begin implementation of a strategy to equitably reduce the total inputs of nitrogen and phosphorus entering the mainstream of the Chesapeake Bay by at least 40% by the year 2000. The target of 40% nutrient reduction was developed by modeling the environmental conditions in the Chesapeake Bay with a two-dimensional, steady-state model. According to modeling efforts, there are 1.35 billion cubic meters of anoxic

water in the bay sometime during the summer and an average of less than 0.5 mg/ℓ of lowest summer dissolved oxygen in the deepest portions of the bay (based on 1985 nutrient loadings). Model results also suggest that a significant improvement in dissolved oxygen in bay bottom waters can be achieved if nitrogen and phosphorus loads are reduced. For example, the model predicts that after full implementation of the 40% reduction goal that the lowest average dissolved oxygen will be 1.67 mg/ℓ and no waters will be anoxic.

Other benefits such as decreased algal biomass are expected as well. Chlorophyll concentrations are expected to decrease (based on model predictions) from 13.6 µg/ℓ to 8.8 µg/ℓ. The reestablishment of rooted aquatic plants and improved fish populations are expected as well but cannot be quantified (Chesapeake Bay Program, 1988). Model predictions are based on the information available in 1985 and the available model did not include a mechanism to account for nutrient loading from sediment.

Since each state had a different mix of point and nonpoint source inputs as well as different programs and policies, each state has a unique plan for nutrient reductions (Chesapeake Bay Program, 1988). This approach allows each state to implement individual strategies that are best suited for that state within the limits of its program constraints, while still reaching the overall goal of 40% reduction at the bay-wide level.

In addition, the reduction target is to be reevaluated by December, 1991 based on modeling, research, and monitoring results.

Program Objectives

The first step in developing a bay-wide monitoring program was to review historical data. Although there were some data shortcomings, the Chesapeake Bay Program was nevertheless able to demonstrate degraded environmental conditions.

Three general program objectives were developed (Magnien and Haire, 1989):

- Quantify the extent and nature of water quality problems
- Determine the response of key water quality variables to management actions
- Develop and test hypotheses on how the Bay ecosystem functions, especially as it relates to anthropogenic stresses and management solutions

Based on eutrophication concerns and the complexity of the Chesapeake Bay, it was also clear that the monitoring network would need to:

- Incorporate physical, chemical, and biological variables
- Collect mainstream and major tributary data
- Collect data consistently over an extended period of time
- Determine the optimal sampling strategy for each variable

As a result of data characterization and development of program objectives, six monitoring program components were formed and are summarized below (Magnien and Haire, 1989 and Chesapeake Bay Program, 1989a):

Chemical/Physical Variables: salinity, temperature, Secchi depth, dissolved oxygen, suspended solids, nutrient species (nitrogen, phosphorus, carbon, and silicon), phytoplankton pigments, heavy metal and organic compounds in surficial sediments

Phytoplankton: species counts, phytoplankton pigments by horizontal and vertical *in vivo* fluorescence, primary productivity, light penetration

Zooplankton: micro (44 µm - 202 µm) and meso (>202 µm) species counts, biomass

Benthic Organisms: species counts, production, sediment characteristics, salinity, dissolved oxygen

Ecosystem Processes: sediment-water column exchange rates of dissolved inorganic nutrients (nitrogen, phosphorus, and silicon) and oxygen, surficial sediment characteristics, deposition rates of particulate matter (total seston, nitrogen, phosphorus, carbon, phytoplankton pigments)

River Inputs: flow, suspended solids, nutrient species (nitrogen, phosphorus, carbon, and silicon), phytoplankton pigments

Variable selection and spatial/temporal intensity were evaluated in order to provide the level of information necessary to support confident management decision making. In general, historical station locations were used as initial station locations and were

"filled-in" with additional stations to provide adequate spatial coverage. Selected sampling frequency was either monthly or bimonthly.

1991 Reevaluation Study

A review of the progress made toward the goals established in the Nutrient Reduction Strategy will be completed by December 1991. Included in this review is the development of a three dimensional dynamic model that will overcome previous limitations and permit more precise estimates.

A2.3 Evaluation of Monitoring Program Performance

The use of statistical power analysis to evaluate expected monitoring program performance is demonstrated below using a subset of data from the Chesapeake Bay Monitoring program. The purpose of evaluating monitoring program performance is not to determine whether the goals of the nutrient reduction program have been met but rather to determine what is the minimum change that the monitoring program can be expected to detect. This information can be used to address a series of questions regarding the adequacy of the monitoring effort. Examples include:

- Are sufficient data being collected to detect a change in ambient nutrient concentrations equal to the expected decrease?
- How long (e.g., number of years) will it take to detect the level of change predicted by the numerical models?

This example includes a description of the data obtained from the Chesapeake Bay Program. Several analytical techniques used to explore the statistical nature of the data are also described. The purpose of these analyses is to identify characteristics of the data that might affect the applicability of statistical tests. Next, statistical tests for trends are described, and results of the analysis of the example data set are presented. Statistical trend analyses were conducted in order to determine whether a trend could be detected. Finally, a series of power analyses are then presented and discussed. It should be noted that the results from the small subset of data used in this demonstration is not necessarily indicative of the expected performance of the Chesapeake Bay monitoring program. An analysis of the nutrient data that has been collected from throughout the bay would be required.

However, this example describes the use of general procedures can be applied to these and other types of monitoring data.

Example Data Set and Sampling Layout

For this example, data were obtained for dissolved oxygen and ammonium as nitrogen from 15 stations. These stations were chosen because they provided adequate spatial coverage as well as having similar sampling methodologies. The station locations (four stations from segment 3, ten stations from segment 4, and one station from segment 5) are shown in Figure A2-1. The principal characteristics of the segments are (Flemer *et al.*, 1983):

Segment 3

- Upstream limit of deep water anoxia
- Moderate salinity (7-13 ppt. mean)
- Two-layer, estuarine circulation driven primarily by freshwater inflow

Segment 4

- Water deeper than 30 feet, oxygen depletion in summer, potentially toxic to fish, crabs, shellfish and benthic animals.
- Mean salinity of 9 to 14 ppt.
- Rich in nutrients

Segment 5

- Influenced by inflow from the Potomac and Patuxent and rich in nutrients.
- Mean salinity of 10 to 17 ppt.
- Subject to summer anoxia and contains most of the deeper Bay waters.

Observations of the water quality random variables are collected bimonthly (monthly during winter months), for a total of 20 samples per year at four station depths. These depths are denoted as surface, above the pycnocline, below the pycnocline, and bottom. When the estuary was not stratified at selected station locations, the pycnocline data were collected at equally spaced distances between the surface and

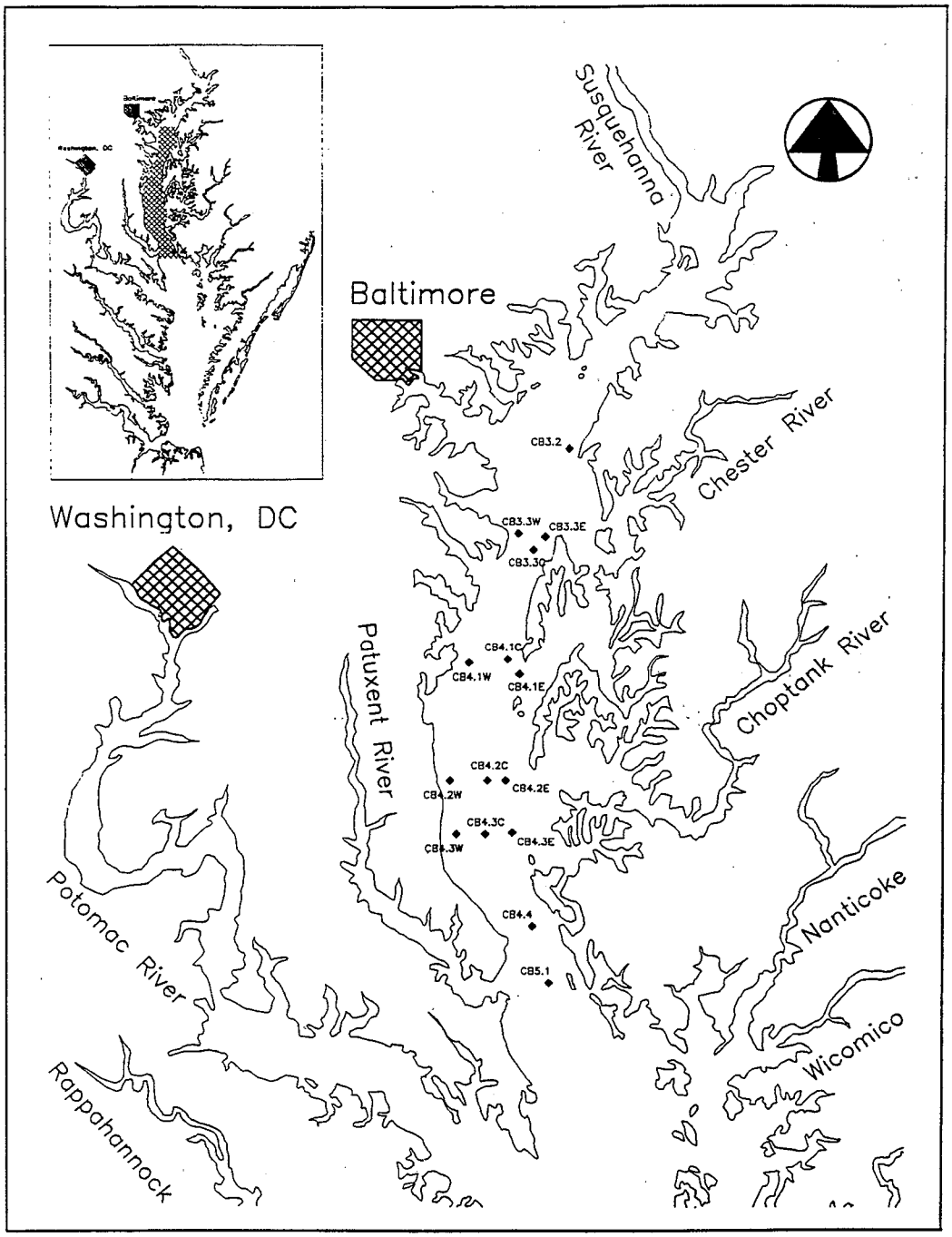

Figure A2-1. Station locations included in data subset of the Chesapeake Bay Ambient Monitoring Program.

bottom. Approximately 66 months (June, 1984 through November, 1989) of data were available for each water quality random variable.

The subgroup of stations from the Chesapeake Bay Program was selected based on the physical grouping provided by Flemer *et al.* (1983). In this example analysis depth is used to group station locations. Further studies could be developed to separately analyze each segment as defined by Flemer *et al.* (1983).

Exploratory Analyses

Before applying a statistical test for trend, several aspects of the time series need to be explored. These include: distributional characteristics (i.e., deviations from normality), the existence of trends, seasonality, and serial correlation.

The choice of the appropriate statistical test will depend on which of these characteristics the data exhibit. The distributions of the data were shown to be non-normal. Time series plots were examined visually for trend and seasonality. A series of monthly box plots for each monitoring variable was made to examine the distribution of monthly data and the seasonal pattern. Each box plot represents that month's data over six years for a group of stations and shows the mean, median, and several percentiles (see Figure A2-2) .

Figures A2-3 and A2-4 show the time series plot for ammonia as nitrogen for the a surface station and dissolved oxygen at a bottom station. The recurring sinusoidal pattern indicates seasonality , a common occurrence in water quality data (Harcum *et al.*, 1990).

Figures A2-5 and A2-6 show the box plots for nitrogen as ammonia and dissolved oxygen. If there was no seasonality, the means for all the months would be approximately the same. However, the monthly mean values vary depending on the month which indicates that there is seasonal pattern.

The data were also tested for serial independence, (i.e., that the value at one timepoint is dependent on the value of the previous timepoint). Most stations were found to exhibit independence.

Statistical Tests for Trend

The choice of statistical test should be selected based on the data characteristics described above. (Overall, the data were not significantly correlated; however, the

data were seasonal and nonnormally distributed.) The Seasonal Kendall tau test was selected since the test accounts for seasonality and is applicable to data that are not distributed normally. Numerous researchers (e.g., Hirsch *et al.*, 1982; Hirsch and Slack, 1984; Berryman *et al.*, 1988; Ward *et al.*, 1988; Camoncho and Haywood, 1988; Taylor and Loftis, 1989; and Harcum *et al.*, 1990) have also suggested that the Seasonal Kendall tau test is an appropriate test for detecting gradual trends with the type of data collected in the Chesapeake Bay Program and is typically more robust over a wider range of conditions than alternative tests.

The Seasonal Kendall tau is a nonparametric test based on ranks. Each data point is assigned rank based on its magnitude (smallest values get the lowest ranks, etc.). The ranks are compared within a season (in this case, a season is a month) across the years (May, 1984, May 1985, etc.) to determine if there is a trend. The results from each season are then tallied and compared to a criterion value obtained from the normal table.

The uneven sampling frequency of the example data set presents some potential problems for the analysis described. Each sampling period (season) cannot be treated equally since more samples were collected in the summer months. Additionally, the

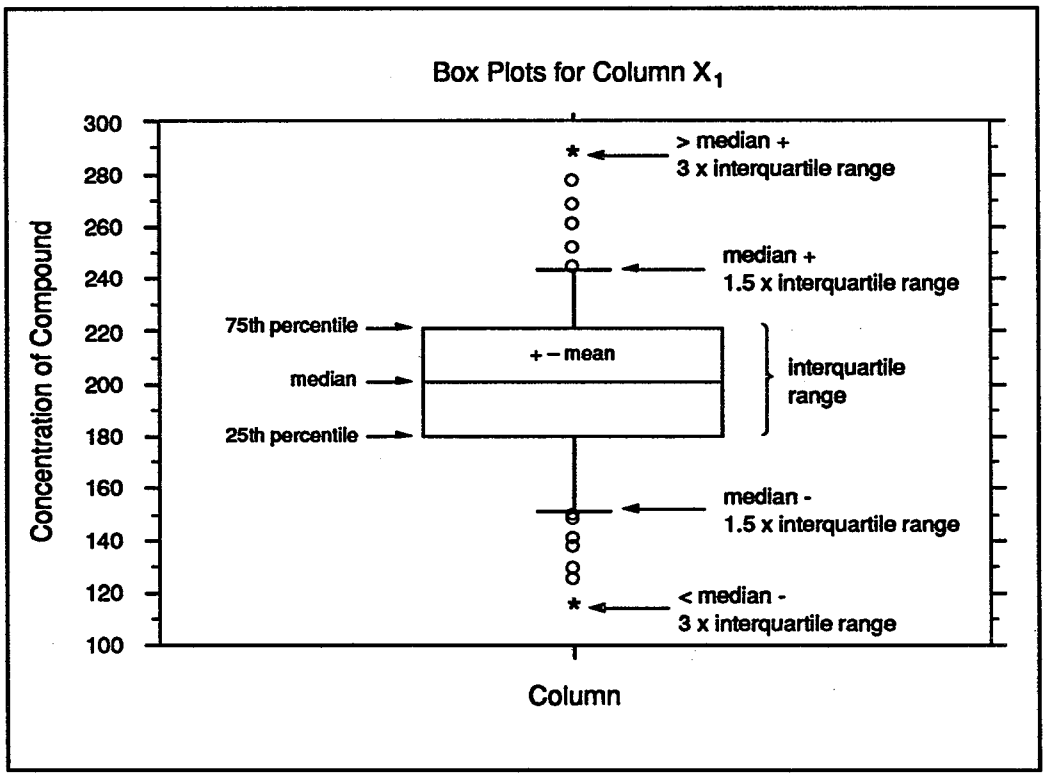

Figure A2-2.
Example box plot.

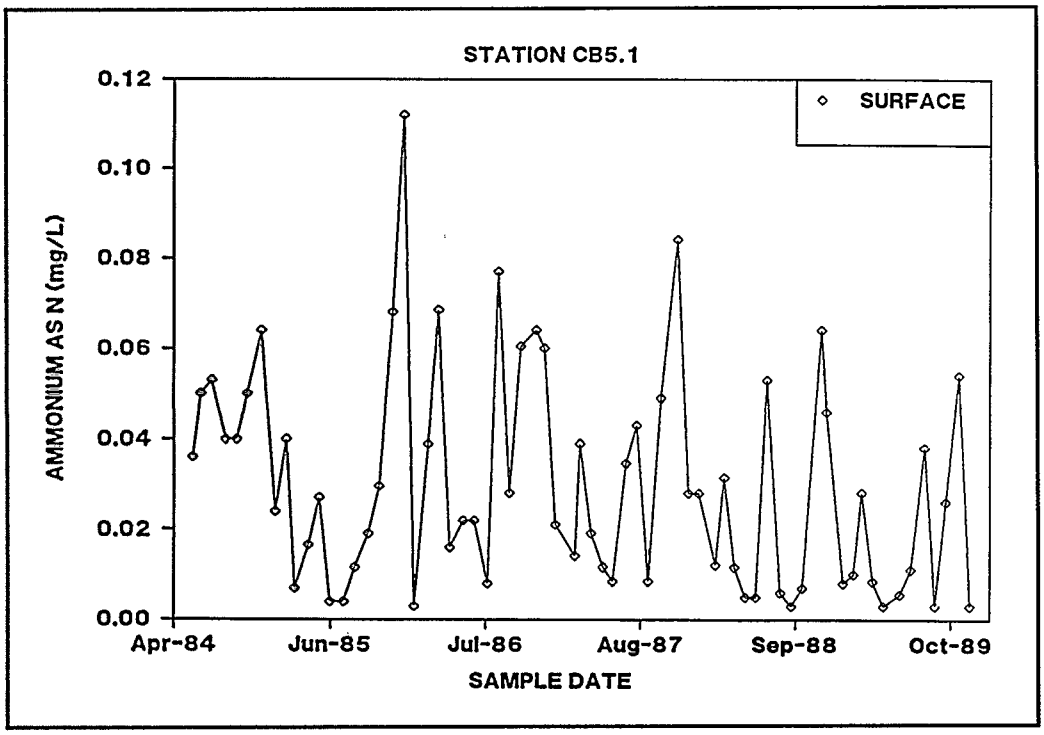

Figure A2-3. Time series for N as NH_3 for surface stations.

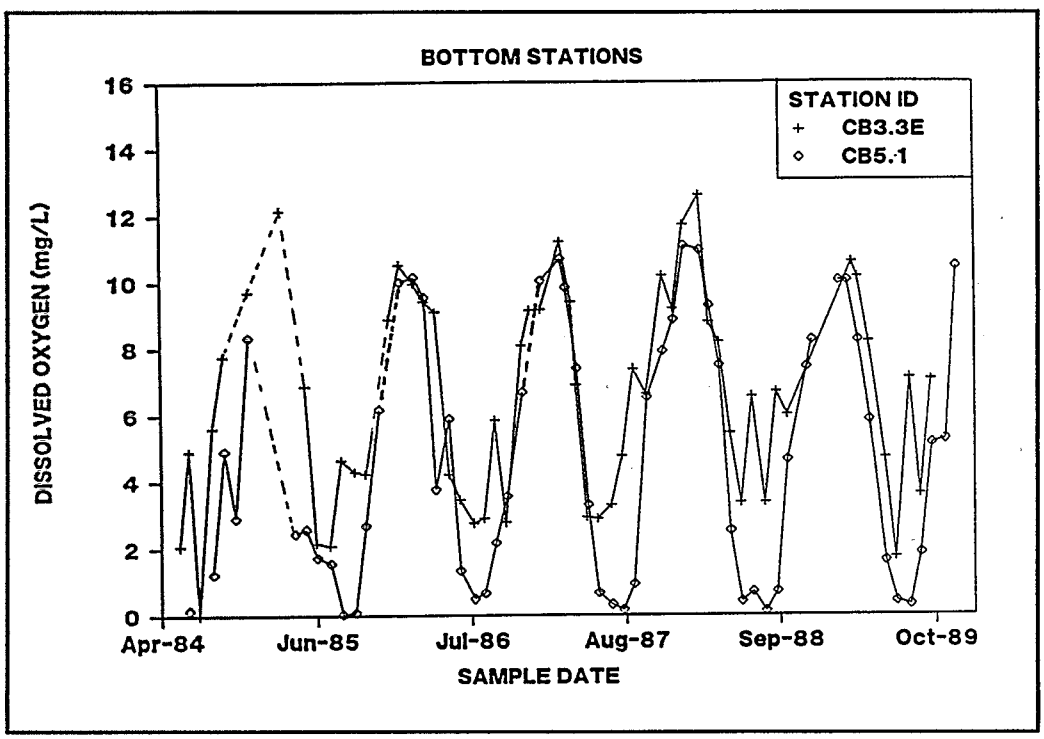

Figure A2-4. Time series for Dissolved O_2 for bottom stations.

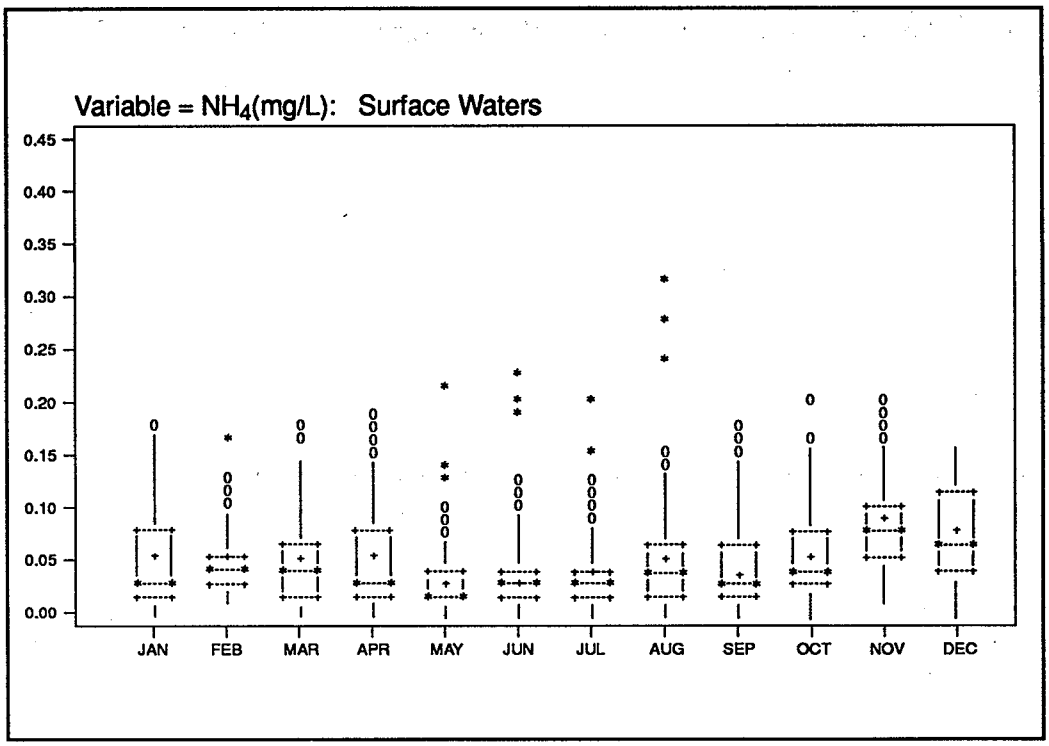

Figure A2-5. Box plot for N as NH_4 for surface stations.

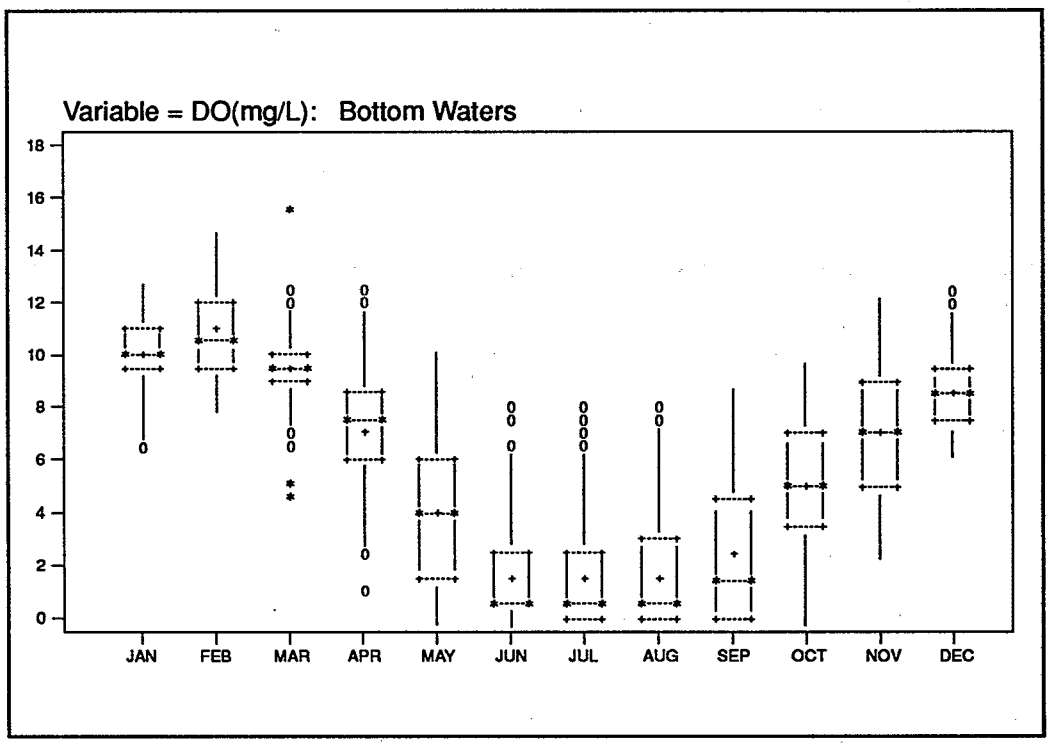

Figure A2-6. Box plot for dissolved O_2 for bottom stations.

likelihood of violating the independence assumption of statistical tests for trends is increased since summer samples were collected more frequently.

Two typical solutions for handling the unequal sampling are 1) averaging or 2) subsampling (e.g., average monthly values to one monthly value or discard one value per month during summer). In this case, past research (Harcum *et al.*, 1990) has shown that averaging data is the preferred method for this type of a monitoring program. For the analyses presented below, average values were computed for 12 seasons (i.e., one value was computed for each month). Equal weight was given for each month for long term trend detection.

For more information about the Seasonal Kendall tau test see Hirsch, *et al.* (1982), Hirsch and Slack (1984), or Taylor and Loftis (1989) .

The results of the Seasonal Kendall tau tests are summarized in Table A2-1. At the 0.10 significance level, several stations showed statistically significant negative trends in ammonium concentrations for both the surface and bottom samples. Positive trends were also detected in dissolved oxygen concentrations in the bottoms waters at two of the fifteen stations analyzed.

Several of the stations shown in Table A2-1 did not demonstrate a statistically significant trend in ammonium or dissolved oxygen concentrations. However, the inability to reject the null hypothesis (i.e., Ho: no trend in measured values) does not justify its acceptance. The level of sampling effort or period of observation may not have been sufficient to detect real trends in the data.

The reader is cautioned that the data preprocessing procedure used here is not appropriate for all applications. Consider a monitoring program composed of monthly observations for the first five years and quarterly observations for the next five years. It is not appropriate to average the monthly observations to quarterly values for comparison with the last half of the data record. Rather, to apply the Seasonal Kendall tau test, one must subsample from the earlier record (discarding two observations per quarter) to create a uniform record. Depending on the exact circumstances, alternative statistical tests may be more appropriate.

Statistical Power Analysis

Statistical power analyses were used to determine the minimum trend that could be detected and therefore to determine the adequacy of the monitoring design.

TABLE A2-1. SEASONAL KENDALL TAU RESULT (α=0.10)

NH_4			O_2		
CB3.2	Surface	No trend	CB3.2	Surface	No trend
CB3.3C	Surface	Negative trend	CB3.3C	Surface	No trend
CB3.3E	Surface	No trend	CB3.3E	Surface	No trend
CB3.3W	Surface	No trend	CB3.3W	Surface	No trend
CB4.1C	Surface	No trend	CB4.1C	Surface	No trend
CB4.1E	Surface	No trend	CB4.1E	Surface	No trend
CB4.1W	Surface	No trend	CB4.1W	Surface	No trend
CB4.2C	Surface	Negative trend	CB4.2C	Surface	No trend
CB4.2E	Surface	No trend	CB4.2E	Surface	No trend
CB4.2W	Surface	No trend	CB4.2W	Surface	No trend
CB4.3C	Surface	Negative trend	CB4.3C	Surface	No trend
CB4.3E	Surface	No trend	CB4.3E	Surface	No trend
CB4.3W	Surface	No trend	CB4.3W	Surface	No trend
CB4.4	Surface	Negative trend	CB4.4	Surface	No trend
CB5.1	Bottom	Negative trend	CB5.1	Bottom	No trend
CB3.2	Bottom	No trend	CB3.2	Bottom	No trend
CB3.3C	Bottom	Negative trend	CB3.3C	Bottom	No trend
CB3.3E	Bottom	No trend	CB3.3E	Bottom	Positive trend
CB3.3W	Bottom	No trend	CB3.3W	Bottom	No trend
CB4.1C	Bottom	Negative trend	CB4.1C	Bottom	No trend
CB4.1E	Bottom	Negative trend	CB4.1E	Bottom	No trend
CB4.1W	Bottom	No trend	CB4.1W	Bottom	No trend
CB4.2C	Bottom	Negative trend	CB4.2C	Bottom	No trend
CB4.2E	Bottom	No trend	CB4.2E	Bottom	No trend
CB4.2W	Bottom	No trend	CB4.2W	Bottom	No trend
CB4.3C	Bottom	Negative trend	CB4.3C	Bottom	No trend
CB4.3E	Bottom	Negative trend	CB4.3E	Bottom	No trend
CB4.3W	Bottom	No trend	CB4.3W	Bottom	No trend
CB4.4	Bottom	Negative trend	CB4.4	Bottom	No trend
CB5.1	Bottom	Negative trend	CB5.1	Bottom	Positive trend

For some statistical tests, it is possible to develop analytical solutions to calculate power based on the statistical characteristics estimated from data (e.g., U.S. EPA, 1987). However for nonparametric tests such as the Seasonal Kendall tau, the standard procedure for computing the test's power is to use Monte Carlo methods.

Monte Carlo simulation methods were used to evaluate the ability of the ongoing monitoring efforts in Chesapeake Bay to detect specified levels of trend in water quality variables.

The analyses were conducted in the following manner:

1) Sampling spaces were defined to exhibit a specified magnitude of trend. The distributional characteristics (e.g., mean and variance) of the sampling population were otherwise similar to the monitoring data obtained from Chesapeake Bay.

2) Random samples from the specified sampling space were generated by computer programs. The simulated sampling frequency was the same utilized in the Chesapeake Bay Monitoring Program. Sampling was simulated for periods of 5 and 10 years.

3) Each sampling experiment was then repeated 500 times.

4) The simulated data in each experiment were analyzed using the Seasonal Kendall Test, and the results of all 500 simulations were summarized to obtain an estimate of the distribution of the test statistic. These results were used to determine the percentage of simulations in which the null hypothesis (no trend) was correctly rejected (i.e, the power of the test).

5) This series of steps was repeated for different magnitudes of trend, and the results were summarized to obtain the plots of the probability of detection versus magnitude of trend shown in Figures A2-7 and A2-8.

In Figures A2-7 and A2-8, four power curves are displayed for ammonia as nitrogen and dissolved oxygen. For each pair of lines (the two solid or the two dashed lines), the line to the right is the projected power for the station with the most variability of the 15 stations and thus least powerful and the line to the left is the station with the least variability and thus the most powerful. The results from the other 13 stations would fall in between these two lines. The solid and dashed lines represent projections for 10 and 20 years of data. As one would expect a significant improvement in trend detection results from ten extra years of data.

Once the power relationships have been developed, selected results may be read from the appropriate figure. From Figure A2-8, for example, dissolved oxygen trends in surface waters on the order of 0.062 to 0.129 mg/ℓ-yr can be detected with 10 years of data at an 80% probability (using the Seasonal Kendall tau test with $\alpha = 0.10$). With 20 years of data and the same group of stations, trends on the order of 0.021 to 0.042 mg/ℓ-yr can be detected with an 80% probability.

The results of these and similar analyses may now be used as a basis to modify, if necessary, the monitoring program. Since this study evaluated a limited selection of

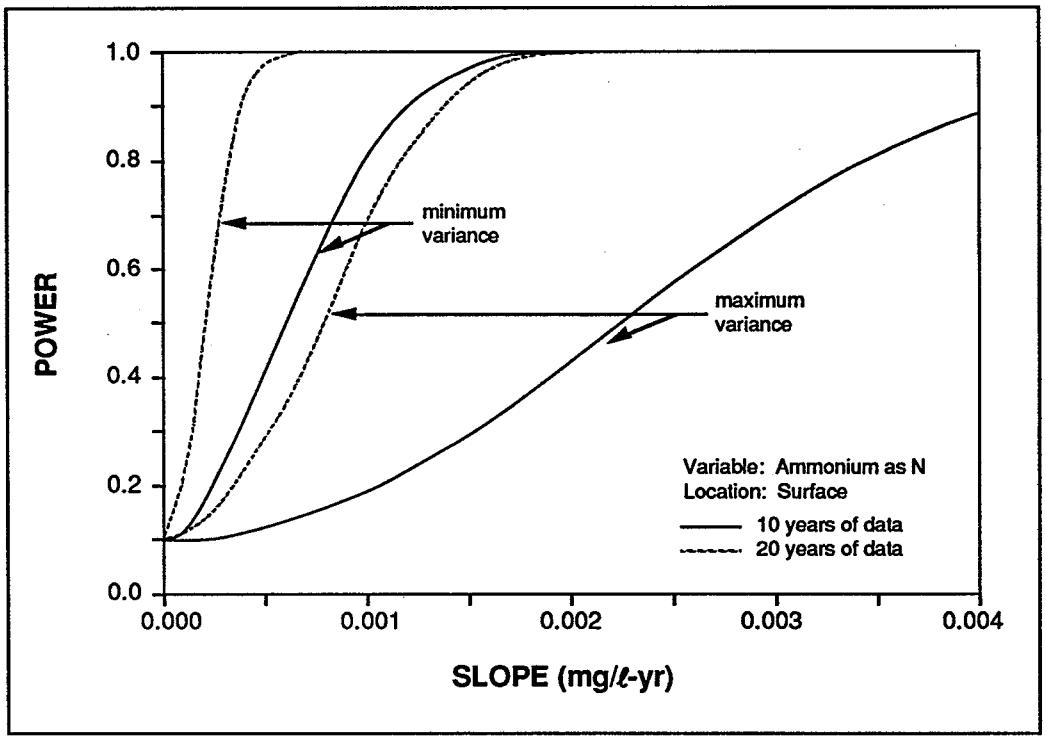

Figure A2-7. Power analysis for N as NH_3 for surface stations.

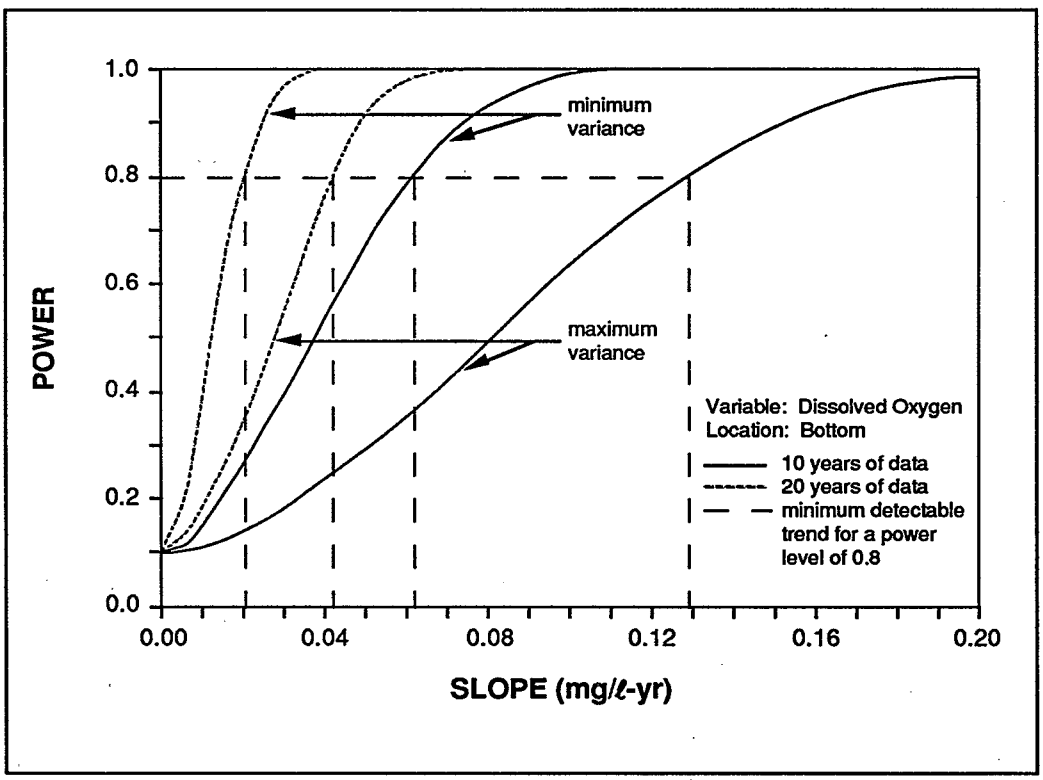

Figure A2-8. Power analysis for dissolved O_2 for bottom stations.

water quality random variables, it is not appropriate to make recommendations for the Chesapeake Bay Program. An extended analysis of other water quality random variables would be needed and balanced against other monitoring objectives, not just long term trend detection.

A2.4 Use of Power Analysis Results

The type of results presented in Section A2.3 can be used to evaluate the performance of individual monitoring program components. By applying this type of analysis to all program components, the management team can then begin to assess the strengths and weaknesses of the overall monitoring effort. This information can be useful in determining where limited financial resources should best be applied.

With the type of information generated in the previous section or similar "what-if" power analysis scenarios, some general questions can be answered:

- Which stations are most effective for detecting trends?
- Which water quality variables are the most useful for detecting trends?

By answering these questions, it is possible to modify the sampling frequency (to collect more or less data), deleting or moving stations, or narrowing or expanding the water quality random variables sampled. By coordinating decisions with the other monitoring objectives, improved evaluation of the physical, chemical, and biological integrity of the estuary is possible.

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Appendix B – Methods

Methods – Introduction

This section identifies and describes sampling and analytical methods that are used in estuarine monitoring programs. The primary purpose of this section is to provide program coordinators and scientific staff of the estuary programs with a basic understanding of the sampling methods as well as information on feasibility and the use of the monitoring program results. As stated in Section 4.1, standardized sampling and analytical protocols or a performance based quality assurance program should be developed for each estuary. This strategy is extremely important to ensure comparability of data. The methods and recommendations described in this Appendix can be used to guide decisions regarding the selection of individual techniques as well as appropriate quality assurance and quality control procedures.

The primary sources of information for these method descriptions were documents developed by the EPA's Office of Marine and Estuarine Protection and the Puget Sound Estuary Program Protocols (described below). Individuals from EPA's Office of Research and Development, the National Oceanographic and Atmospheric Administration, the U.S. Fish and Wildlife Service, university research laboratories and ongoing National Estuary Programs were also contacted for information on latest developments and ongoing research.

B.1 Methods Chapter Format

The methods that are described in this section (Table B-1) are grouped for presentation into four categories: water quality, sediment quality, biological resources and human health risks.

The format that is used to describe the selected sampling and analytical methods is shown in Table B-2. Following a brief description of the monitoring method and a definition of terms, the rationale for monitoring is described in terms of what information will be generated and how that information can be used to address the goals and evaluate the success of the CCMP. Major monitoring design issues such as selection of species for monitoring or selection of temporal and spatial sampling strategies are addressed under the topic of monitoring design considerations. Selected existing laboratory QA/QC and analyses methods are described. Statistical design considerations include method-specific statistical techniques to evaluate expected monitoring program data. The data use section provides guidance for data interpretation and use in addressing issues of concern. All information presented for each monitoring program component is summarized. Each chapter emphasizes the importance of coordinating monitoring program components to assure an effective, cost-efficient monitoring program.

As part of the task of summarizing available information, emphasis is placed on providing references to existing documents. Currently, there is a major cooperative

TABLE B-1. SAMPLING METHODS DESCRIBED

Water Quality
1. Water Column Physical Characteristics
2. Water Column Chemistry

Sediment Quality
3. Sediment Grain Size
4. Sediment Chemistry

Biological Resources
5. Plankton
6. Aquatic Vegetation
7. Benthic Infauna Community Structure
8. Fish Community Structure
9. Fish and Shellfish Pathobiology

Human Health Risks
10. Bioaccumulation
11. Bacterial and Viral Pathogens

effort underway within EPA, the U.S. Army Corps of Engineers, the National Oceanic and Atmospheric Administration, and the National Bureau of Standards to develop a Compendium of Methods for Estuarine and Marine Environmental Studies. The draft document describes methods for nutrient measurements in seawater (U.S. EPA, 1990b). Additional sections are under development. Other important sources of monitoring program guidance include documents developed by the Office of Marine and Estuarine Protection for implementation of the 301(h) permit program. These documents (Table B-3) provide useful information on sampling equipment, field and laboratory methods, statistical techniques, and the design and implementation of marine and estuarine monitoring programs. The Puget Sound Estuary Program, in a 2-year effort, developed standardized protocols for measuring selected environmental variables in Puget Sound (U.S. EPA, 1986-1991). These protocols have been developed for the monitoring topics shown in Table B-4. The process of developing these regionally standardized protocols is described by Becker and Armstrong (1988).

This methods section is also intended to provide a summary of available information and to address the most important issues associated with the design and implementation of the monitoring program. Issues common to all monitoring methods include quality assurance/quality control (QA/QC) and statistical design. These two important issues are discussed below.

TABLE B-2. FORMAT OF METHODS SECTION

Introduction
- description of method
- definition of terms

Rationale
- types of information generated
- applicability of information

Monitoring Design Considerations
- monitoring design issues
- guidance for the implementation of appropiate monitoring design strategies

Existing Analytical Methods
- brief overview of available laboratory and data analysis
- advantages / limitations of methodologies

QA / QC Considerations
- selected QA / QC protocols

Statistical Design Considerations
- statistical design strategies
- guidance for the implementation of appropriate statistical design

Data Use
- use / interpretation of monitoring results
- presentation of data
- integration

Summary and Recommendations

Literature Cited and References

TABLE B-3. TECHNICAL SUPPORT AND GUIDANCE DOCUMENTS

Monitoring Program Development, Implementation and Evaluation

- Design of 301(h) Monitoring Programs for Municipal Wastewater Discharges to Marine Waters. 1982, EPA 430/9-82-010.
- Ecological Impacts of Sewage Discharges on Coral Reef Communities. 1983, EPA 430/9-83-010.
- Initial Mixing Characteristics of Municipal Ocean Discharges. 1985, EPA 600/3-85/073a.
- Summary of U.S. EPA-Approved Methods, Standard Methods, and Other Guidance for 301(h) Monitoring Variables. 1985, EPA 503/4-90-002.
- Analytical Methods for U.S. EPA Priority Pollutants and 301(h) Pesticides in Estuarine and Marine Sediments. 1986, EPA 503/6-90-004.
- Evaluation of Survey Positioning Methods for Nearshore Marine and Estuarine Waters. 1987, EPA 430/9-86-003.
- Framework for 301(h) Monitoring Programs. 1987, EPA 430/09-88-002.
- Guidance for Conducting Fish Liver Histopathology Studies during 301(h) Monitoring. 1987, EPA 430/9-87-004.
- Quality Assurance and Quality Control (QA/QC) for 301 (h) Monitoring Programs: Guidance on Field and Laboratory Methods. 1987, EPA 430/9-86-004.
- Recommended Biological Indices for 301(h) Monitoring Programs. 1987, EPA 430/9-86-002.
- Guide for Preparation of Quality Assurance Project Plans for the National Estuarine Program. Interim Final. 1988, EPA 556/2-8-001.

TABLE B-3. TECHNICAL SUPPORT AND GUIDANCE DOCUMENTS (continued)

- A Simplified Deposition Calculation (DECAL) for Organic Accumulation Near Marine Outfalls. 1987, EPA 430/09-88-001.
- Technical Support Document for ODES Statistical Power Analysis. 1987, EPA 430/9-87-005.
- Evaluation of Differential Loran-C for Positioning in Nearshore Marine and Estuarine Waters. 1988, Tetra Tech., Inc., Draft Report, EPA Contract No. 68-C8-001.

Bioaccumulation Monitoring Guidance Series

- Bioaccumulation Monitoring Guidance: 1) Estimating the Potential for Bioaccumulation of Priority Pollutants and 301(h) Pesticides Into Marine and Estuarine Waters. 1985, EPA 503/3-90-001.
- Bioaccumulation Monitoring Guidance: 2) Selection of Target Species and Review of Available Bioaccumulation Data. 1985, EPA 430/9-86-005.
- Bioaccumulation Monitoring Guidance: 3) Recommended Analytical Detection Limits. 1985, EPA 503/6-90-005.
- Bioaccumulation Monitoring Guidance: 4) Analytical Methods for U.S. EPA Priority Pollutants and 301(h) Pesticides in Tissue from Estuarine and Marine Organisms. 1986, EPA 503/3-90-001.
- Bioaccumulation Monitoring Guidance: 5) Strategies for Sample Replication and Compositing. 1987, EPA 430/9-87-003.

TABLE B-4.
MONITORING TOPICS INCLUDED IN PUGET SOUND ESTUARY PROGRAM PROTOCOLS

- General QA/QC Considerations for Collecting Environmental Samples
- Measuring Conventional Sediment Variables
- Conducting Laboratory Bioassays
- Station Positioning
- Measuring Metals in Water, Sediment, and Tissue Samples
- Microbiological Studies
- Measuring Organic Compounds in Sediment and Tissue Samples
- Sampling and Analyzing Subtidal Benthic Macroinvertebrate Assemblages
- Fish Pathology Studies
- Measuring Conventional Water Quality Variables and Metals in Fresh Waters
- Sampling Soft-Bottom Demersal Fishes by Beach Seine and Trawl
- Measuring Conventional Marine Water Column Variables
- Marine Mammal Tissue Sampling and Analysis

B.2 Quality Assurance/Quality Control (QA/QC) Considerations

The purpose of Quality Assurance is to ensure that the environmental monitoring efforts result in data of known and acceptable quality. There are two types of quality assurance plans: the Quality Assurance Program Plan (QAPP) and the Quality Assurance Project Plan (QAPjP).

Quality Assurance Program Plan. Development of a QAPP is important in establishing the overall framework for implementation of quality assurance within a specific estuary program. It establishes the quality assurance policy, overall procedures and responsibilities.

Quality Assurance Project Plan. Quality Assurance Project Plans must be prepared for all projects involving environmentally-related data as a requirement of both EPA and National Estuary Program policies. The QAPjP is a recognized tool for providing the detailed information needed to ensure the reliability and comparability of data collected during a project. Guidance on developing a QAPjP is provided in U.S. EPA (1988c). Examples of some key considerations are provided in Table B-5.

Data Quality Objectives

A major part of the QA effort is establishment of data quality objectives (DQOs). DQOs are specific, integrated statements and goals developed for each data or information collection activity to ensure that the data are of the required quantity and quality. DQOs should specify the desired sensitivity of sampling methods, timing and

TABLE B-5.
KEY TOPICS TO BE ADDRESSED
IN ESTUARY PROGRAM QUALITY ASSURANCE PLANS

- Instructions for proper sample preservation and storage
- Chain of custody documentation
- Source of standard reagents
- Source or preparation of quality control samples
- Frequency of analysis of quality control samples
- Data Quality Objectives
- Analysis of QC samples
- Review of results
- Corrective action when unsatisfactory results are obtained in the analysis of QA/QC samples
- Calibration and maintenance of equipment

location of sampling, and numbers of samples to be collected. One function of establishing DQOs, as discussed in Section 2.3, is to ensure that monitoring/ data collection studies yield data adequate to meet their intended uses. DQOs are also used to delineate QA/QC programs specifically geared to the data collection activities to be undertaken. Development of DQOs usually consists of three processes: 1) decision definition, 2) data use and needs identification, and 3) data collection program design. DQOs should be continually reviewed during data collection activities and any needed corrective action may be planned and executed to minimize problems before they become significant.

DQOs play an important role in the selection of sample collection, sorting, and analysis methods. In fact, the selection of monitoring methods should be driven by DQOs. Any future modifications to monitoring protocols should be considered only after these new methods meet established data performance criteria. Data collected with different methods should not be compared unless information exists which supports such comparisons.

The following sections provide a discussion of existing monitoring methods. These methods provide a starting point for considering monitoring methodologies. New, cost-effective methods are sure to develop for certain disciplines in the coming years. A key consideration when deciding whether new methods are to be incorporated into the monitoring program, is whether they meet the QA/QC program's performance criteria. QA/QC considerations in the following sections will primarily discuss sample collection, processing, storage, and analysis.

QC Samples

Additional samples, either collected in the field or prepared from standard reference materials are used to check for field or laboratory contamination, test laboratory accuracy, test field or laboratory reproducibility, and assess natural variability. There are four basic types of QC samples that should be submitted with each set of sediment or water quality samples (Table B-6). Although the frequency with which these samples are collected will vary among programs, each should be collected at least once for every 20 samples or sampling event, whichever is more frequent.

Data from existing monitoring programs will often be utilized in an estuary program. Therefore, data will frequently be collected by a number of agencies following different sampling and analytical strategies. Under such conditions

TABLE B-6.
QC SAMPLE TYPES

Travel (Trip) Blank	assess cross-contamination during handling and transport
Rinsate Blank	assess contamination due to incomplete cleaning of sampling equipment
Method Blank	assess sample contamination due to reagents/sample handling in the laboratory
Field Replicate	assess variability in sampling technique or laboratory handling

data comparability becomes a major QA/QC consideration. Data comparability must be evaluated based on both field and laboratory sources of variability. Individual Estuary Programs will need to address this aspect of QA on a case by case basis. The Chesapeake Bay Program, for example, has implemented a coordinated split sample program to assess comparability among sampling and analytical operations basinwide (Bergstrom, 1990; Chesapeake Bay Program, 1991). Although the specific protocols followed in this program may not be suitable for all Estuary Programs, the use of a coordinated QA/QC sampling strategy to assess data comparability is generally applicable.

B.3 Statistical Design Considerations

Prior to the collection of data, it is necessary to determine how the data will be used to support decisions. It is further recommended that analytical performance criteria (e.g., minimally acceptable Type I and Type II error) be defined to establish quantitative expectations for the monitoring program. The link between data, performance and decision-making should be specified *a priori* to ensure that appropriate data, and spatial and temporal coverages are addressed by the monitoring plan.

Selection of the number of replicates is an important component of program design. The use of power analyses, examining alternative sampling and compositing strategies, will lead to an effective monitoring design strategy. These statistical techniques may mitigate the high costs of collecting and processing samples. As a general recommen-

dation, equal numbers of samples should be collected whenever possible to simplify statistical analyses.

Composite Sampling

Composite sampling consists of mixing two or more replicates and/or samples. The chemical analysis of a composite sample provides an estimate of an average contaminant concentration for locations and/or times comprising the composite sample (see space and time bulking below). Advantages of the composite sampling strategy are:

- provides cost-effective strategy when individual chemical analyses are expensive
- results in a more efficient estimate of the mean at specified sampling locations.

Because of the reduced sample variance, composite sampling may result in a considerable increase in statistical power. If the primary objective of a monitoring program is to determine differences in contaminant concentrations among sampling locations, composite sampling is an appropriate strategy.

Space-bulking consists of sampling from several locations and combining samples into one or more composite samples. Time-bulking involves taking multiple samples over time from a single location and compositing these samples. A disadvantage of using space- and/or time-bulking strategies is that significant information concerning spatial and temporal heterogeneity may be lost.

Compositing of tissues provides a means to analyze bioaccumulation when the tissue mass of an individual is insufficient for the analytical protocol. If mixing species, tissue composites are likely to be composed of different proportions of species, and different numbers, age, and sex of individuals. These differences among composites tend to confound whether patterns of tissue residue concentrations are due to differences in locations or interspecific differences in bioaccumulation. Mixing of species is not recommended because of these confounding effects.

Composite sampling is not recommended if the objective of the monitoring program is to determine compliance with specified sediment contaminant concentration limits, since this sampling method does not detect the true range of sediment contaminant

concentrations in the environment. The adoption of composite strategies will depend upon the objectives of individual monitoring programs.

Statistical Power

The large degree of temporal and spatial variability observed for many ecosystem state and rate variables requires collection of sufficient replicate samples to ensure an accurate description of the measure of interest. However, increases in replication increase sample processing costs. Power analyses assist in the allocation of sampling resources (stations, replication, and frequency) with regard to program finances and design (Sokal and Rohlf, 1981).

Power analyses may be applied to determine the appropriate number of sample replicates, and/or subsamples in a replicate composite, required to detect a specified difference (U.S. EPA, 1987b). The number of replications required to detect a specified minimum difference is a function of the statistical power and the variance in the data. Power analyses require a prior knowledge of the variability in the data. A best guess or variation observed in historical data are often used initially in the design of the monitoring program.

For composite samples, statistical power increases with the increase of the number of subsamples in each replicate composite sample (U.S. EPA, 1987c). However, there exists a diminishing return of statistical power with the addition of successive subsamples to each composite. For composites of greater than ten replications, the increase of power is negligible given typical levels of data variability.

To improve the power of a statistical test, while keeping the significance level constant, increase the sample size. However, due to constraints in cost and time imposed by the CCMP this option may not be available. Power analyses have shown that for a fixed level of sampling effort, a monitoring program's power is generally increased by collecting more replicates at fewer locations. The number and distribution of sampling locations required to evaluate the effectiveness of the CCMP will be depend upon the size and complexity of the estuary.

Power-Cost Analyses

The relative power of one design with respect to another is more meaningful when the relative costs of implementing alternative designs are taken into consideration. Analyses of power-costs are fundamental in selecting appropriate sample/replicate number,

sample location, and sampling frequency (Bros and Cowell, 1987; Cuff and Coleman, 1979; Ferraro *et al.*, 1989; Millard and Lettenmaier, 1986).

A cost function which describes the cost per replicate sample is required. Power-cost formulations for parametric statistical analyses are of the form:

$$\text{Power-cost}_i = (1 - \beta_i) / (c_i \times n_i)$$

where **i** is a replicate sample collection and analysis scheme, **c** is the cost per replicate sample, **n** is the number of replicate samples, and β_i is the Type II error. Costs will depend upon sample collection, sorting, and analysis equipment and protocols. Type II error (ß) is a function of the number of replicate samples and the variance detected by a sampling and analysis scheme. The measured variance may be influenced by the sampling equipment used and the unit replicate sample size (e.g., as the unit replicate area increases, variance increases).

Total costs are usually fixed. Therefore, iterations of power-cost formulations for various sample processing scenarios (e.g., sample collection, sorting, and analysis schemes) and their comparisons will result in the selection of the appropriate monitoring methodology. Sokal and Rohlf (1981) provide a series of formulas for calculating the most efficient sampling design for a given cost.

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B1.0 Water Column Physical Characteristics

B1.1 Rationale

Physical characteristics of the water column are typically monitored to evaluate estuarine water quality. The objective of monitoring these physical characteristics is to identify and detect spatial and temporal variations in water quality. These results may be used to characterize the water column, to monitor estuarine hydrodynamics, and to verify estuarine hydrodynamic models.

Many chemical and biological processes in the environment are affected, directly or indirectly, by the physical characteristics of the environment (Thomann and Mueller, 1987). Collection of physical data also provide ancillary information necessary in evaluating chemical and biological data.

B1.2 Monitoring Design Considerations

Monitoring schemes for physical characteristics usually involve *in situ* methods. Data concerning the water column's physical characteristics can be collected at relatively inexpensive costs; the expense of different methods is generally governed by the level of automation. Typically, the location and frequency of sampling water column physical characteristics are determined by other, more expensively collected water column parameters. However, it is essential to standardize the monitoring design in order to ensure the comparability of monitoring data throughout the program.

Sampling Depths

When using bottle sampling techniques, samples for temperature, salinity, and turbidity should be taken at a minimum of four depths in the vertical profile: 1) one meter below the surface, 2) one meter above the bottom, 3) one meter above the pycnocline, and 4) one meter below the pycnocline. If the waters are too shallow or no stratification occurs, it would be appropriate to take the latter two samples at evenly spaced distances between the top and bottom samples. However, features of water masses recorded in the historical data (i.e., historical profiles of salinity, temperature, and turbidity) and the collection of other data types (e.g. plankton community structure and water chemistry) should be considered when establishing sample depths (Pond and Pickard, 1983).

When using *in situ* methods [e.g., Conductivity-Temperature-Depth (CTD)] temperature and salinity measurements should be taken at one meter intervals over the entire depth profile (to within 1 meter of the surface and bottom). Little additional cost is incurred for this detailed characterization of the water column once the CTD is deployed. In areas of high stratification, a smaller interval would be appropriate.

A consistent vertical sampling design is recommended in order that comparisons may be conducted between data collected at different locations and over time. For example, a set of depths may be specified by the monitoring design; at a minimum, any sampling of the water column must include data from this set of specified depths.

Spatial and Temporal Scale

Generally, as the scale and complexity (spatially and/or temporally) of circulation increases, more meters need be deployed to adequately describe the environment. The need for macroscale and detailed descriptions of circulation will depend upon the objectives of the CCMP and the physical structure of the estuary. Circulation patterns and tidal dynamics may result in the establishment of fronts (i.e., regions of steep gradients) and the accumulation of pollutants and organisms (Dustan and Pinckey, 1989; Pinckey and Dustan, 1990).

The length of the sampling period will be determined by monitoring objectives. If descriptions of seasonal variations and/or high frequency natural events (e.g., tides, storms, etc.) are necessary, measurements should be collected over a period not less than two years (Fischer *et al.*, 1976). Frequent sampling in fish and shellfish areas, and other biologically sensitive areas may be required in order to detect rapid changes in those habitats which may endanger these resources.

Moored instrument arrays provide a cost-effective method of continual sampling over long periods of time (from days to months) for many physical characteristics. More commonly used at remote offshore locations, subsurface arrays can provide unattended long-term data collection in estuarine environments for both physical and chemical parameters. Water depth, velocity (speed and direction), temperature, and conductivity or salinity are the most commonly measured characteristics. Estimates of total suspended solids and chlorophyll can be recorded also, using turbidometers, transmissometers or fluorometers. Dissolved oxygen concentrations can also be measured. Sensors capable of *in situ* measurements of nutrient and trace contaminant concentrations are under development.

Moored arrays have the potential to provide synoptic coverage at pre-determined intervals, which can range from minutes to days. The longer the interval between data recordings, the longer the array can function without replacing the power supply (usually batteries) or the recording medium (cassette tape or computer memory chips). Real-time telemetry of data to a shore station is possible with the more sophisticated (and more expensive) instrumentation packages. More commonly, the instrument array is retrieved at regular intervals and the collected data post-processed.

Moored arrays that are deployed for long periods of time are susceptible to many types of interference. Within estuaries, deployment must take into consideration the impacts of strong tidal flows, vessel traffic, unintentional interference from sub-surface nets, fishing lines and anchor lines, and from intentional interference, such as theft and vandalism.

B1.3 Existing Analytical Methods

Methods available for monitoring physical characteristics include instruments that range from simple mercury-filled thermometers that provide one observation of temperature to state-of-the art CTD (Conductivity-Temperature-Depth) equipment that provide vertical profiles of salinity and temperature. Methods (and/or equipment) discussed in this section are summarized in Table B1-1.

Temperature

Surface temperature measurements may be made with any good grade of mercury-filled centigrade thermometer, or a thermistor taking care not to expose the water sample to the sun or to the evaporative cooling effect of the wind (APHA, 1989). For measuring subsurface temperatures the basic instrument is the "protected reversing thermometer"; a mercury-in-glass thermometer which is attached to the water sampling bottle (Pond and Pickard, 1983).

Bathythermographs (BTs) consist of a thermistor and an electronic pressure transducer. Wires extending from the BT to the vessel relay measures of temperature and pressure. BTs may be operated from stationary or slow moving (< 4 knots) vessels. Expendable bathythermographs (XBTs) are based on tracking an expendable, free-falling bathythermograph. The system is designed to operate from a stationary or moving vessel (< 15-20 knots) typically in deep waters. Wires extending from the free-falling transducer to the vessel relay temperature and depth recordings. The wires are cut when the probe reaches its maximum depth. BTs and XBTs do not record measures of salinity. If it has been determined that density is not related to salinity, bathythermographs may be a cost-effective tool. Monitoring of temperature and pressure using XBTs is higher in cost since the probe is lost after each vertical profile.

Conductivity-Temperature-Depth (CTDs) meters are the tool of choice, since density measures are typically dependent on salinity, temperature, and depth (see Density below). Measures of density are important in identifying and tracking the movements of water bodies and in determining the depth at which a water mass will settle at equilibrium. CTDs coupled with a computer can continuously integrate temperature and conductivity measurements with depth. CTD/computer provides real-time read-

TABLE B1-1. LIST OF METHODS AND EQUIPMENT

Temperature

- mercury-filled centigrade thermometer
- thermistor
- reverse thermometer
- bathythermograph
- CTD

Salinity

- Knudsen titration
- conductivity cells (CTD instruments)

Density

- direct (hydrometers, pycnometers, magnetic float densimeters)
- indirect (function of salinity, temperature, and pressure)

Depth

- fathometer
- meter wheel
- hydrostatic pressure
  - bourdon tube
  - strain gauge

Turbidity

- light transmissometer
- Secchi disc
- nephelometer
- turbidimeter

Current Measurements

- Eulerian method (speed and direction)
  - mechanical
  - electromagnetic
  - acoustic
- Lagrangian method
  - drogues
  - surface drifters
  - seabed drifters

Dye Studies

- fluorometers

outs which allow immediate modifications in CTD position and ensures comprehensive water column coverage during the survey.

Salinity

The Knudsen titration method determines chlorinity by titration with a standard silver nitrate solution and salinity is then computed from a standard formula (Pond and Pickard, 1983). This titration method is practical but not convenient to use on board a ship (APHA, 1989).

Conductivity cells are used for *in situ* use and a variety of such instruments are now available from several manufacturers (Pond and Pickard, 1983). A CTD unit, consisting of conductivity, temperature, and depth sensors, is lowered through the water on the end of an electrical conductor cable which transmits the information to recording units on board ship (UNESCO, 1988).

Density

Density is an important identifying property of sea water because there is no process, except mixing, by which this property changes below the surface (Pickard and Emery, 1982). Direct measures of density are slow (Pond and Pickard, 1983). Devices such as hydrometers, pycnometers, and magnetic float densimeters may be used when great accuracy is not required.

In practice, the density – or the specific gravity – of sea water can be determined from temperature, salinity, and pressure. Temperature, salinity, and pressure are measured directly and the density is read from tables which have been prepared from laboratory determinations (e.g., Eugene, 1966). Algorithms for automated data processing have been developed by UNESCO and are applicable for salinities as low as 2 ppt (Fofonoff and Millard, 1983). For lower salinities, see Hill *et al.* (1986).

Depth

The simplest method of determining the depth of an instrument is to pass the wire attached to the instrument over a meter wheel with a known circumference and a counter. In calm conditions and negligible currents, the length of wire passed over the meter wheel will be the actual depth.

Depth may also be estimated using a bourdon tube moving the slider of an electrical potentiometer or a strain-gauge pressure transducer. The hydrostatic pressure exerted

on gauge or bourdon tube may then be converted to a depth (Pickard and Emery, 1982).

The water depth at a particular station is typically determined with acoustics. Depth-sounders have accuracies of 0.3 to 0.7 ft (Clausner *et al.*, 1986).

Turbidity

To determine the transmission of visible light through the water, the simplest device is the Secchi disc (Pond and Pickard, 1983). Measurements of Secchi disc depth are probably the most widely used means of estimating light penetration. The Secchi disc is easy to use and can be used to estimate the attenuation coefficients for collimated and diffuse light. As a result, the depth of the euphotic zone may be estimated. Secchi disk readings vary with the observer because of interpersonal differences in visual ability and, therefore, caution must be exercised when comparing Secchi disk readings taken by different observers.

Turbidity is a measure of the absorption and scattering of light by particulate matter (plankton and suspended sediments) as it passes through water. Measurements may be either *in situ* or remote. In remote measurements, water samples are obtained from discrete depths and then analyzed on deck or in the laboratory with a device such as a nephelometer. This method of sampling is quick, but is labor intensive and is restricted to sampling only at those depths where water samples were taken.

In situ sampling involves passing a light transmissometer or turbidimeter (usually as part of a CTD system) through the water column. This provides for much higher sampling rates and less labor. To correlate data taken from different instruments all values should be expressed consistently as a percent transmittance for a standard pathlength.

Nephelometry is the preferred method for measuring turbidity. Because nephelometers measure light that is refracted at 90° as it passes throght the sample, they are more sensitive, and effective over a wider range of turbidities than other turbidimeters. The 90° detection angle is also less sensitive to variations in particle size. Turbidity measured using nephelometric methods is expressed nephelometric turbidity units (NTU).

Current Measurements

There are two basic ways to describe fluid flow, the Eulerian method in which the velocity (i.e., speed and direction) is stated at every point in the fluid, and the Lagrangian method in which the path followed by each fluid particle is stated as a function of time.

Eulerian Methods – Mechanical meters include Savonious rotors, ducted impellers, drag inclinometers, and propeller-type meters. Although Savonious rotors have been used for many years in estuarine work, they are not suited for operation in shallow water environments which are exposed to wave and swell activity. Ducted impeller current meters have been designed to reduce the problems associated with current measurements in the presence of waves (Brainard and Lukens, 1975). Davis-Wellar propeller type meters are also designed to operate in the presence of waves.

Electromagnetic current meters measure the instantaneous horizontal and vertical velocity components at a flow sensor. The characteristics of these meters make them suitable for use in the presence of waves and shallow water (McCullough, 1977). Most recent electromagnetic current meters are equipped with several probes to measure other physical characteristics of the receiving water.

Acoustic current meters determine the current velocity by emitting a short acoustic pulse and measuring the Doppler frequency shift of the backscattered signal reflected from small, passively transported particles in the water column (Appell and Curtin, 1990; Appell and Woodward, 1986). This instrument allows for non-intrusive, remote measures of current velocity throughout the water column from a depth of 3 to over a 1000 meters. The current meter can be placed on a ship and sampling conducted while the ship is underway, thereby allowing for a synoptic measurement of the current field over a large area with the use of only one instrument. Acoustic current meters are suitable for use in the presence of waves but the accuracy of the measurements decreases close to the water surface. These instruments are also expensive and require highly trained technicians to maintain them.

Another technology employs the use of land based radar systems sending out electromagnetic waves and measures the reflected energy from the surface waves (Appell and Curtin, 1990; Appell and Woodward, 1986). Since this system is set up on the shore it only works out to a few dozen kilometers offshore and only measures the current velocity in the top meter of the water column. This system does have the advantage in that a ship is not needed to deploy or retrieve the instrument.

Lagrangian Methods – Most drogues are drogue-buoy systems consisting of a small marker buoy which is tracked at the surface and a larger submerged drogue portion which is set at the desired depth by connecting line between the two portions (U.S. EPA, 1982). The drogue portion must be weighted and ballasted so that the drogue assembly has sufficient negative buoyancy to keep both the drogue and connecting line in their intended vertical orientation, and keep the buoy mast upright. Drogues are intended to passively drift with the currents at a specified depth. In reality, some error is introduced in the drogue trajectories by wind drag on the exposed portion of the

marker buoy, by the relative surface current drag on the submerged portion of the surface buoy, and, for deep drogues with long lines, by the relative current drag on the connecting line. If possible, it is best to avoid drogue studies under high wind conditions, especially when measuring lower current speeds in deeper waters. Drogues can be used from just a couple of days to many months and can be tracked by satellite.

Most drogues can be classified into one of the following four categories: 1) Parachute drogues, 2) Cruciform drogues, 3) Window Shade drogues, and 4) Cylindrical drogues. Of these types, parachute and cruciform drogues are the most widely used (U.S. EPA, 1982).

Surface drifters are used to measure the average path of currents at the surface. Drift bottles and drift cards are the types most commonly used. Vertical drift bottles and drift cards are useful for evaluating the movement of effluent which reaches the surface. Horizontal drift cards, which float on the surface, may be used to determine the potential movement of surface slicks due to oils or other floatables which form a surface film. Because these movements are influenced largely by the wind, horizontal drift cards should be released under several different conditions (Grace, 1978).

Seabed drifters measure the average path of currents near the sea floor. They are useful for determining the fate of waste materials subject to transport by bottom currents. This includes settleable solids and any portion of the effluent which remains near the bottom. The drifters provide information on the net movement of a waste field along the bottom, including where the waste field may reach the shoreline, and a rough estimate of how long it may take. If a sufficient number of drifters are recovered, they may indicate areas of possible shoreline contamination (Grace, 1978).

B1.4 QA/QC Considerations

All environmental monitoring programs should have a written and approved quality assurance project plan (U.S. EPA, 1987a). Water column sampling devices should be checked for proper functioning prior to the survey. It is prudent to have a backup sampler onboard the survey vessel in case the primary sampler is found to be unsuitable during the cruise.

Tables B1-2 and B1-3 lists sample preservation and storage requirements and recommended methods for salinity and temperature (U.S. EPA, 1987a). When several methods are available, the selection should be made by comparing the accuracy and precision of the candidate methods for the parameter range expected at the site. All monitoring programs should follow manufacturers recommended calibration procedures.

TABLE B1-2. RECOMMENDED SAMPLE PRESERVATION AND STORAGE REQUIREMENTS

Parameter	Volume Required (ml)	Container	Preservative	Holding Time
Salinity	240	G (with paraffined corks air tight)	None	1 hour (Longer if properly sealed)
Temperature	1,000	P,G	Determine on site	No holding

References: Standard Methods (APHA, 1989) and Methods for Chemical Analysis of Water and Wastes (U.S. EPA, 1983)

Note: P = Polyethylene, G = Glass

Each temperature-measuring instrument should be calibrated against a precision thermometer certified by the National Bureau of Standards at least every week during sampling (U.S. EPA, 1986-1991). It is recommended that calibration be conducted more frequently when temperature variation is suspected to ensure that readings reflect environmental differences, not instrument drift.. Temperature probes may, at best, be accurate to within one-tenth of a degree Celsius. The required sensitivity will depend on the amount of natural variability in the environment and the amount of difference which needs to be detected. Temperature probe systems are rarely linear over large temperature ranges and must be checked against research grade laboratory thermometers (APHA, 1989).

Salinity probe systems offer moderate accuracy but should be cross-checked by discrete water samples analyzed by induction type laboratory salinometers (APHA, 1989). Two standard determinations should be made before the start of each series of samples. In addition, one standard sample should be analyzed, to monitor instrument

TABLE B1-3.
RECOMMENDED ANALYTICAL METHODS

Parameter	Method	Precision	Minimum Detection	Significant Digits Desired
Salinity	Induction Salinometer or titration, (APHA, 1989)	titration:	1 ppt	4
Temperature	Bathythermograph or Thermometric. EPA Method 170.1. (APHA, 1989)	± 0.05°C	–	3

The water column, being a 3-D medium, requires greater numbers of samples to be collected (*vs.* a 2-D medium) in order to be adequately described.

drift. It is recommended that duplicate determinations be made for at least 10 percent of the samples analyzed (U.S. EPA, 1987a).

Many multi-probe *in situ* measurement systems incorporate depth measurements by use of pressure transducers. The accuracy and precision of such system must be periodically checked. A pressure calibration can be done in a laboratory pressure chamber. Daily calibration may be done in calm waters of a known depth (e.g., inside a lagoon or a marina). Precision is determined by multiple measurements at the same depth. Accuracy is evaluated by comparison to measurements made with a heavily weighted line.

Instruments that measure turbidity require regular calibration. *In situ* instruments are most accurately calibrated by the use of primary standards, usually a carefully prepared suspension of formazin or other approved standard. Transmissometer type instruments can be calibrated by comparison to solutions of known total suspended solids. These solutions should approximate the turbidity of the water that is to be sampled. For other instruments, including those nephelometers or turbidity meters that

measure discrete samples, either on deck or in the laboratory, calibration using an approved primary standard suspension is recommended (APHA, 1989). Calibration in all cases should be conducted at the start of each series of analyses and after each group of ten successive samples. Duplicate analyses should be conducted on at least ten percent of the total number of samples (U.S. EPA, 1986-1991).

Furthermore, the following recommendations should be considered:

- current meters should be calibrated before and after each major deployment of instruments
- UNESCO (1988) presents details on the calibration of CTD instruments
- fluorometer calibration prior to use with a series of prepared concentrations, blanks and spikes are recommended quality control checks (Wilson, 1986).

B1.5 Statistical Design Considerations

In general, one expects to find temporal and spatial patterns in physical characteristics. The primary purpose of monitoring the physical characteristics of the water column is to describe these patterns (e.g., range, seasonal variations) and to determine the primary physical mechanisms affecting the monitored area.

As a result, statistical comparisons between sampling locations and sampling periods are not common except for turbidity. In this instance, the reader is referred to the *Water Chemistry* section for analytical approaches that would be appropriate.

Temporal Analyses

Tidal and seasonal variability in the distribution and magnitude of several water column physical characteristics are typically observed (Day *et al.*, 1989). Time series analyses – e.g., temporal autocorrelation, spectral analyses – may be necessary to examine the effects of these cyclical influences and/or to filter out these cyclic forcing functions in order to examine long-term temporal trends.

Graphical Representations

The physical characteristics data described in this section are typically summarized in graphical form; the most common being temperature-depth profiles, salinity-depth profiles, and temperature-salinity plot (T-S plot). Alternatively, horizontal sections (e.g., contour maps) of properties are useful for displaying geographical distributions.

Multiple physical characteristics may be displayed by placing one graph above the other. More details about analyzing characteristic diagrams can be found in Pickard and Emery (1982).

Current data can be displayed in numerous fashions including stick plots, a time series of rectangular components, and horizontal maps (Pickard and Emery, 1982). These figures are typically plotted after periodic components are removed through spectral analysis leaving only the residual non-periodic components.

B1.6 Use of Data

Physical characteristics may be used to determine water column stability, to characterize estuarine conditions, and to identify existing and potential problems affecting habitats of fish and sensitive life-stages of marine organisms (e.g., upwelling areas). Turbidity can be used to estimate the reduction of light transmittance. Reduction of the depth to which sunlight penetrates, i.e., turbidity increase, causes a decrease in the compensation depth of phytoplankton resulting in reduced biological growth (Thomann and Mueller, 1987).

Physical characteristics data are used to calibrate and validate hydrodynamic models (Davis, 1988; Leighton, 1988; U.S. EPA, 1982). If mathematical models will be used to assess the effects of ambient physical characteristics, the monitoring program should be designed to provide the model with the required boundary condition data. It may also be necessary to collect meteorological data (e.g., wind speed and direction, temperature, pressure) for modeling efforts since estuary circulation can be affected by wind effects.

Data concerning salinity, temperature, and currents may be used in interpreting the temporal and spatial variability of distributions of particular species and benthic infaunal communities.

B1.7 Summary and Recommendations

Rationale

- Most chemical and biological processes in the marine environment are affected, directly or indirectly, by physical characteristics of the marine environment (Thomann and Mueller, 1987)

- Physical characteristics may be used to evaluate hydrodynamics and to provide ancillary information to interpret other water chemistry variables

Monitoring Design Considerations

- Temperature, salinity, density, turbidity, and depth (depth of sample and depth to bottom) should be collected at a sufficient number of depth intervals to obtain a realistic picture of the water column profile for temperature and salinity at each sampling location; in most cases, a 1 m interval will provide the necesssary resolution

- Sampling frequency should be determined by the estuary program's requirement for detecting seasonal or high frequency events (e.g. tides, storms, etc.); measurements should be collected over a period of not less than two years in order to examine seasonal effects (Fischer *et al.*, 1976)

- More frequent sampling in fish and shellfish areas, and other biologically sensitive areas may be required

Existing Analytical Methods

- Temperature measurements may be made with:
  - mercury-filled centigrade thermometer
  - thermistor
  - reversing thermometer
  - bathythermograph
  - CTD instrumentation

- Salinity measurements are preferably made with conductivity cells (CTD instruments)

- Density measurements are to be calculated from salinity, temperature, and pressure

- Depth measurements are preferably made with hydrostatic pressure techniques

- Turbidity measurements may be made with:
  - light transmissometer
  - Secchi disc
  - nephelometer
  - turbidimeter

- Current measurements may be made with:
  - Eulerian methods – e.g., propeller-type meters, electromagnetic meters, acoustic-doppler meters, etc.
  - Lagrangian methods – e.g., drogues, surface drifters, etc.

QA/QC Considerations

- Calibrate all equipment regularly

- Blank and replicate analyses are recommended QC checks

Statistical Design Considerations

- The primary purpose is to describe what the patterns (e.g., range, seasonal variations) of physical characteristics are and to determine the primary physical mechanisms affecting the monitored area

- Statistical comparisons between sampling locations and sampling periods are not common except for turbidity

- Common graphical summaries include:
  - temperature-depth, salinity-depth profiles, and temperature-salinity (T-S) plots
  - horizontal sections (i.e., contour maps) of properties with multiple physical characteristics displayed by placing one graph above the other

- Current data can be displayed with stick plots, a time series of rectangular components, and horizontal maps (Pickard and Emery, 1982)

Use of Data

- Physical characteristics are used to determine hydrodynamics and to provide ancillary information to interpret other variables

- Turbidity can be used to estimate the reduction of light transmittance. Reduction of the depth to which sunlight penetrates, due to turbidity increase, can reduce biological community growth (Thomann and Mueller, 1987)

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B2.0 Water Column Chemistry

Government agencies and research institutions rely on monitoring data to evaluate the quality of water bodies. However, there are a number of problems associated with data collected over the years. One of the most significant problems is the lack of comparability due to inconsistent choice of methods and poor technique and/or quality control practices. Commonly employed EPA laboratory methods were intended to detect pollutants at much higher concentrations than those usually found in natural waters. Alternative analytical methods are at present being used or developed in conjunction with existing NEP monitoring programs (e.g., Puget Sound, Chesapeake Bay) and EPA methods continue to be updated. The best choice of individual analytical protocols will be dependent on site- or area-specific conditions, such as contaminants of concern, biological communities, and the level of anthropogenic impact. If methods other than those approved by the EPA are required to achieve a desired sensitivity, it should be demonstrated that these methods result in equivalent or better data quality with respect to accuracy and precision. Several EPA documents (e.g., 1987a, 1987b) discuss quality assurance/quality control (QA/QC) protocols, sampling gear, and recommended laboratory methods. The Compendium of Methods for Marine and Estuarine Environmental Studies (U.S. EPA, 1990), currently in preparation, includes a number of method variations for nitrogen species, phosphorus, and chlorophyll.

B2.1 Rationale

Estuarine waters represent an important habitat for many commercially, recreationally, and ecologically important organisms. These waters also represent the medium in which food, larvae, nutrients, and contaminants may be transported throughout the estuary. The objective of monitoring water column chemistry is to detect and describe spatial and temporal changes of contaminants in and around the estuary.

The results of monitoring of water column contaminant concentrations can provide a spatial and temporal record of anthropogenic perturbations to the estuary. The results may be used to monitor rates of recovery following environmental interventions, to evaluate the condition of aquatic habitats, and to provide early warnings of potential impacts to the estuarine ecosystem.

B2.2 Monitoring Design Considerations

The decision of which chemicals to include in the monitoring program is not simple. One approach is to request analysis for all chemicals on the EPA-defined Priority Pollutant, Hazardous Substance, or Target Compound/Analyte Lists. However, detection in the environment does not always correlate with biological effects. Furthermore, many of the chemicals that pose risks to estuarine and marine systems are hydrophobic. Water column monitoring for hydrophobic chemicals may indicate

concentrations that are several orders of magnitude below concentrations in sediments and biota. Unless compelling site-specific conditions warrant otherwise, water column monitoring for hydrophobic chemicals is not recommended. Limitations in analytical methodology, modelling techniques, and toxicological data restrict the usefulness of the resulting data. In some cases, levels of contaminants which are of concern to the health of organisms are below the lower limit of detection of the best available analytical methodology.

Selection of sampling locations and choice of sampling techniques are crucial decisions to be made before the sampling effort is initiated. Random sampling may not detect "hot spots" associated with point source discharges. Too much sampling close to a point source discharge will, however, bias the evolution of overall ambient conditions.

Water column sampling design must consider not only the horizontal location of the sampling stations (addressed in Section 4.0), but also the vertical location within the water column and the time of sample collection. Because the majority of chemical analyses are performed, by necessity, on discrete samples, consideration should be given to the number of depths at which samples are collected. Obviously, such decisions will be based on site-specific characteristics, such as water depth, contaminants of concern, suspected sources, and the sensitivity or importance of local biota. Costs related to the laboratory analyses should also be considered when determining the number of samples desired.

The time of sample collection is a site- and target compound-specific consideration which can strongly influence data comparability. For example, dissoved oxygen concentrations fluctuate diurnally especially in shallow brackish water. Some contaminant concentrations may vary as a result of tidally driven freshwater or saltwater intrusions. The effects of seasonal ambient wind patterns, or river flows and runoff events associated with seasonal weather patterns should be considered in sample design.

Water Column Sampling Equipment

Water column samples are frequently collected using water bottles. They are simple devices, usually consisting of a cylindrical tube with stoppers at each end and a closing device that is activated by an electrical signal. Each samples a discrete parcel of water at any designated depth. Multiple samplers are fixed on a rosette frame in order that several depths may be sampled during one cast and/or that replicate samples may be

collected at a particular depth. The most commonly used bottle samplers include the Kemmerer, Van Dorn, Niskin, and Nansen samplers (U.S. EPA, 1986-1991). Alternatively, a pump may be used to sample the water column.

Caged Organisms

The California Mussel Watch Program and the National Oceanic and Atmospheric Administration (NOAA) Status and Trends Program have employed the use of caged transplant mussels to monitor bioaccumulation of toxic chemicals over space and time (Ladd *et al.*, 1984; Goldberg *et al.*, 1978). Caged sentinel species offer several advantages:

- they concentrate contaminants (up to 10^2-10^5 fold vs. estuarine waters), facilitating laboratory analyses of contaminant concentrations
- they provide a means of temporally integrating water quality conditions
- they provide an assessment of biological availability of water column contaminants
- they may be deployed and maintained in a number of diverse locales

The Long Island Sound Estuary Program has shown that chemical residue analyses on caged indicator species appears to be a promising approach for identifying sources of pollution (U.S. EPA, 1982).

However, the disadvantages of caged organisms include:

- the cost of transplanting organisms
- the possibility of loss of the cage/buoy system
- the possibility of introducing a "nuisance" species

Species have different bioaccumulation potentials for various contaminants. It is recommended that multiple sentinel species be deployed in order to ensure that a number of contaminants are sufficiently evaluated and a comprehensive characterization of water contaminants be conducted. Unfortunately, formulations which would allow comparisons of bioaccumulation data between different species and/or different

tissues types have not yet been developed; comparisons of tissue burdens among species is not acceptable. Thus, it is essential that monitoring design elements be standardized to allow for comparisons among estuarine studies.

B2.3 Existing Analytical Methods

Chemical Analyses

Questions to be considered during the choice of appropriate analytical methods include the parameters of interest, desired detection limits, sample size requirements or restrictions, methods of preservation, technical and practical holding times, and matrix interferences (especially from saline water). Saline waters cause additional problems due to matrix effects. D'Elia *et al.* (1989) discuss the common analytical problems encountered during monitoring analyses of water samples.

Selection of appropriate methods should consider a compromise between full-scan analyses, which are economical but cannot provide optimal sensitivity for all compounds, and alternate methods that are more sensitive for specific compounds, but can result in higher analytical costs. The Chesapeake Bay program, for example, has used alternative methods or modified EPA approved methods to achieve greater sensitivity in the analyses of several nutrient and trace metal parameters. Possible interferences include suspended solids, metal ions, and residual chlorine. A list of existing analytical techniques is presented in Table B2-1.

Dissolved Oxygen – The titrimetric, or Winkler, method is the first method of choice for the measurement of dissolved oxygen (U.S. EPA, 1983). The membrane electrode method is recommended for samples containing interferents such as sulfur compounds, chlorine, free iodine, color, turbidity, or biological flocs, or when continuous monitoring is planned (U.S. EPA, 1983).

Nutrients – Nutrients such as ammonia nitrogen, total Kjeldahl nitrogen, nitrate-nitrite nitrogen, and total phosphorus are typically determined by spectrophotometric measurements using a segmented continuous flow analyzer where samples and reagents are continuously added in sequence separated by air bubbles and pumped through glass tubing (U.S. EPA, 1983). Automated analyses can be accomplished in less time than would be required by manual methods because each analysis is not carried to completion, but is brought to the same stage of development and exposure by the timing of the stream flow through the system.

Ammonia nitrogen is determined by treating the samples with alkaline phenol and hypochlorite to produce indophenol blue which is intensified with sodium

TABLE B2-1. LIST OF EXISTING ANALYTICAL TECHNIQUES

DISSOLVED OXYGEN	
• Winkler Titration	U.S. EPA Method 360.2
• Membrane Electrode	U.S. EPA Method 360.1
NUTRIENTS	
• Continuous Flow Spectrophotometry	
Ammonia Nitrogen	U.S. EPA Method 350.1
Total Kjeldahl Nitrogen	U.S. EPA Method 351.2
Nitrate-Nitrite Nitrogen	U.S. EPA Method 353.2
Total Phosphorus	U.S. EPA Method 365.4
• Fluorescence Spectrometry	
Chlorophyll a	APHA (1989) 1002.G.2
• High Performance Liquid Chromatography (HPLC)	
TRACE METALS	
• Atomic Absorption Spectrophotometry (AA)	U.S. EPA Method 7000 series
- flame	
- graphite furnace	
- cold vapor	U.S. EPA Method 7470
- gaseous hydride	U.S. EPA Methods 7060 and 7740
• Inductively Coupled Plasma Emission Spectrometry (ICP)	U.S. EPA Method 6010
ORGANICS	
• Gas Chromatography (GC)	
- with electron capture detection (GC/ECD)	U.S. EPA Method 8080
- with mass spectrometry (GC/MS)	U.S. EPA Methods 8240 and 8270
• Liquid Chromatography	
- with mass spectrometry (LC/MS)	

nitroprusside. Standard solutions should be made up using substitute sea water to approximate the matrix of the samples (U.S. EPA, 1983). Total Kjeldahl nitrogen is defined as the sum of free ammonia and organic nitrogen compounds which are converted to ammonium sulfate under the conditions of digestion (U.S. EPA, 1983). Sulfates react with nitrogen compounds of biological origin, but may not convert nitrogenous compounds of some industrial wastes. Two digestion methods have been used for determining Kjeldahl nitrogen, the "block method" (U.S. EPA Method 351.1) and the "Helix method" (U.S. EPA Method 351.2). The block method is recom-

mended because the Helix method underestimates Kjeldahl nitrogen due to incomplete digestion of the sample. The Chesapeake Bay Program has developed adjustment equations to compensate for these differences and increase accuracy when the Helix method is used (Computer Sciences Corporation, 1992). For the determination of nitrate-nitrite nitrogen, a filtered sample is passed through a granulated copper-cadmium column to reduce any nitrate to nitrite. The nitrite is then transformed to a highly colored azo dye which is measured spectrophotometrically.

The Chesapeake Bay Program has modified U.S.EPA Method 350.1 for ammonia nitrogen determination by increasing the flow-through cell length to 50 mm and using a different set of buffers. These modifications have provided the greater sensitivity required for estuarine monitoring. This program also recommends the following set of procedures to determine organic nitrogen rather than U.S. EPA Method 351.2 for Total Kjeldahl Nitrogen: 1) total nitrogen is determined either by combustion using a CHN analyzer, or by oxidation of all nitrogen forms to nitrate for determination as nitrate-nitrogen, 2) analysis of nitrate, nitrite, and ammonia nitrogen by colorimetry, and 3) calculation of organic nitrogen as the difference between the total nitrogen and the sum of the nitrate, nitrite, and ammonia values.

Total phosphorus is determined by heating the sample in the presence of sulfates, then cooling the sample and measuring phosphorous spectrophotometrically.

The fluorometric method for chlorophyll a is more sensitive than the spectrophotometric method. The sample is subjected to an excitation wavelength and the fluorescence is measured at a second emission wavelength (APHA, 1989). The greatest uncertainty in the method is the choice of reference standard. HPLC is the most accurate of the methods used for the analysis of chlorophyll (since all forms can be separately quantified), however it is also the most expensive.

Trace Metals – The choice of analytical method for trace metal analysis is determined by the required detection limit. ICP allows the simultaneous measurement of several elements. However, the achievable detection limits are usually not as low as those obtained by graphite furnace or hydride AA. The combination of AA and ICP is the recommended analytical method for detection of metals since no technique is best for all elements. Greater sensitivity in trace metal analyses can frequently be achieved through extraction and concentration using chelation extraction. For example, although cold vapor AA is the recommended technique for the analysis of mercury (U.S. EPA, 1986-1991), greater sensitivity is achieved if mercury is first concentrated and then analyzed by the gold amalgam/mercury technique.

Graphite furnace AA is more sensitive than flame AA or ICP, but is more subject to matrix and spectral interferences, which result in potential QC problems during the analyses and uncertainties in the resulting data. Because of the lower concentrations which can be detected by graphite furnace AA, particular caution must be taken with regard to laboratory contamination. The concentration of each element is determined by a separate analysis, making the analysis of a large number of contaminant metals both labor-intensive and relatively expensive compared to ICP. However, AA methods may be cost-effective for the analysis of a few metals over a large number of samples.

Semivolatile Organic Compounds – Analysis of semivolatile organic compounds involves a solvent extraction of the sample, cleanup of the extract, GC separation, and quantitation (U.S. EPA, 1986-1991). There are two GC/MS options for detecting extractable organic compounds: internal standard technique and isotope dilution. The isotope dilution technique is recommended because reliable recovery corrections can be made for each analyte with a labeled analog or a chemically similar analog. This method is, however, more expensive and less widely employed than the internal standard technique, which is the current method of choice in the Contract Laboratory Program. Mass spectrometry provides positive compound identification by comparison of both retention time and spectral patterns with standard compounds.

The identification of pesticides and PCBs can be made by GC/ECD analysis (U.S. EPA, 1986-1991). GC/ECD provides greater sensitivity (lower detection limits) relative to GC/MS, however GC/ECD does not provide positive compound identification. Since the identity of a compound is determined solely by matching retention time with that of a standard, confirmation of pesticides and PCBs on a second GC column is required for confidence in the reliability of the qualitative identification of the compound. When a compound is present at a high enough concentration, its identity should be confirmed by the use of GC/MS.

Glass capillary GC achieves a much higher degree of resolution in the analysis of PCB congeners than the standard packed column methods; however, few labs regularly use this specialized technique.

Volatile Organic Compounds – Analysis of volatile organic compounds also involves GC analysis and quantification (U.S. EPA, 1986-1991). The purge and trap GC/MS technique is commonly employed for detecting volatile organic compounds in water. As in the case of semivolatiles, GC/ECD may be used to achieve lower detection limits, although this introduces a level of uncertainty to the qualitative identification of compounds. The isotope dilution technique is recommended if the DQOs of

the monitoring program require accurate quantitation of each compound. This technique, however, carries additional analytical time and expense.

B2.4 QA/QC Considerations

Sample Collection

Sampling Gear – The primary criterion for an adequate sampler is that it consistently collect undisturbed and uncontaminated samples. Water column sampling devices should be inspected for wear and tear leading to possible sample leakage upon ascent. It is prudent to have a backup sampler onboard the survey vessel in case the primary sampler is found to be unsuitable during the cruise.

In the field, sources of contamination include sampling gear, lubricants and oils, engine exhaust, airborne dust, and ice used for cooling samples. Samples designated for chemical analyses require all sampling equipment – e.g., siphon hoses, scoops, containers – be made of noncontaminating material and be cleaned appropriately prior to use. Potential airborne contamination – e.g., stack gases, cigarette smoke – should be avoided. Furthermore, samples and sampling containers should not be touched with ungloved fingers.

Sample Handling and Storage

Splitting water samples for chemical analyses should only be conducted with noncontaminating tools under "clean room" conditions. Recommended container materials, sample sizes, preservation techniques, and storage lifetimes for all compounds of concern in water are summarized in Table B2-2.

Laboratory Analyses

Laboratory performance as measured by method QA/QC protocols should be used to evaluate and select appropriate analytical methods. Changes to laboratory protocols should only be considered if new protocols meet established performance criteria and provide accuracy and precision equivalent to or greater than EPA approved methods. Suggested QA/QC protocols and analytical performance criteria are discussed below.

QA/QC reports should describe the results of quantitative QA/QC analyses, as well as other elements critical to the laboratory analyses of water, sediments, and tissues to ensure proper interpretation of the results. It is recommended that these reports be recorded and stored in a database for future reference.

TABLE B2-2.
SAMPLE PRESERVATION AND STORAGE PARAMETERS

	Sample		Storage	
Analyte	Container[a]	Size	Preservative	Lifetime
Nutrients				
Ammonia	P,G	500 ml	H_2SO_4 pH<2[b]	28 days
Nitrate	P,G	100 ml	H_2SO_4 pH<2[b] refrigerate	48 hrs
Nitrate + Nitrite	P,G	200 ml	H_2SO_4 pH<2[b]	28 days
Nitrite	P,G	100 ml	H_2SO_4 pH<2[b]	48 hrs
Organic	P,G	500 ml	H_2SO_4 pH<2[b] refrigerate	days
Total or dissolved metals (except Hg)	P,G,TFE[c]	1 l	HNO_3 pH <2	6 mo
Total or dissolved Hg	G,TFE	250 ml	HNO_3 pH <2	8 days
Particulate Metals	P,G	1 gal	[d]	[d]

[a] P = linear polyethylene, G = borosilicate glass, TFE = tetrafluoroethylene.

[b] It is recommended that samples be analyzed as soon as possible.

[c] If aliquot for Hg taken from this 1 liter sample, cannot use linear polyethylene.

[d] Samples should be filtered as soon as possible and always within 24 h. Workshop attendees recommend that filtering be done shipboard rather than in lab on shore.

Field QA/QC Checks – Travel (trip) blanks will indicate whether any contamination occurred in the field or during shipping of samples. Rinsate blanks check for contamination due to inadequate cleaning of field equipment.

Field splits, treated and identified as separate samples, may be sent to the same laboratory for analysis or one sample sent to a "reference" laboratory for comparison. Standard reference material may be placed in a sample container at the time of collection and sent "blind" to the laboratory.

Instrument QA/QC Checks – Calibration standards should be analyzed at the beginning of sample analysis, and should be verified at the end of each 12-hour shift during which analyses are performed (U.S. EPA, 1987b). The concentrations of calibration standards should bracket the expected sample concentrations, otherwise sample dilutions or sample handling modifications (e.g., reduced sample size) will be required.

Method QA/QC Checks – Analysis of preparation blanks should be conducted to demonstrate the absence of contamination from sampling or sample handling in the laboratory. At least one method blank must be included with each batch of samples and should constitute at least 5% of all samples analyzed.

Spike recovery analyses are required to assess method performance for the particular sample matrix. Spike recoveries serve as an indication of analytical accuracy, whereas analysis of standard reference materials measure extraction efficiency. Recommended control limits include 75-125 percent recovery for spikes, and 80-120 percent recovery for the analysis of standard reference materials.

Replicates must be analyzed to monitor the precision of laboratory analyses. A minimum of 5% of the analyses should be laboratory replicates. The control limits are ±20 percent variation between duplicates. Triplicates should be analyzed on one of every 20 samples or on one sample per batch if less than 20 samples are analyzed.

Laboratory performance and calibration should be verified at the beginning and end of each 12-hr shift during which analyses are performed.

Currently, there exists no universally agreed upon convention for reporting detection limits of analytical procedures. Table B2-3 lists definitions of various detection limits used by the American Chemical Society's Committee on Environmental Improvement (CEI). The IDL does not address possible blank contaminants or matrices interferences and is not a good standard for complex environmental matrices. The LOD and LOQ account for blanks, but not matrix interference. The MDL provides high statistical confidence but, like the LOQ, may be too stringent. Detection limits must be specified and reported to ensure adequate data quality and comparability among protocols.

Detection limits should be low enough, as practically possible, to allow detection of contaminants of concern and subsequent statistical analyses.

TABLE B2-3.
DEFINITIONS FOR SELECTED LIMITS OF DETECTION

Instrument Detection Limit (IDL)	the smallest signal above background noise that an instrument can detect reliably.
Limit of Detection (LOD)	the lowest concentration level that can be determined to be statistically different from the blank. The recommended value for LOD is 3s, where s is the standard deviation of the blank in replicate analyses.
Limit of Quantitation (LOQ)	the level above which quantitative results may be obtained with a specified degree of confidence. The recommended value for LOQ is 10s, where s is the standard deviation of blanks in replicate analyses.
Method Detection Limit (MDL)	the minimum concentration of a substance that can be identified, measured, and reported with 99 percent confidence that the analyte concentration is greater than zero. The MDL is determined from seven replicate analyses of a sample of a given matrix containing the analyte.

B2.5 Statistical Design Considerations

The statistical analysis of water chemistry data typically involves estimating summary statistics and testing for spatial and temporal trends. Summary statistics are useful for spatial displays (e.g., plume contours), load estimations, and general reporting. Testing for trends is useful for evaluating whether water chemistry conditions have improved or degraded over time or space. In comparison to other variables (see Bioaccumulation Methods), it is not common to collect replicated or composite data except for QA/QC procedures. This is the usual case since most monitoring designs typically opt for collecting water chemistry data more frequently or at more stations.

Summary Statistics

To summarize data, the analyst typically estimates statistics for central tendency (e.g., mean, median) and variability (e.g., standard deviation, interquartile range) of the data. Uncertainty should be indicated by reporting estimates with confidence limits or percentiles (Ward and Loftis, 1986). Data may also be summarized graphically with plots (e.g., box and whisker plots, time series plots, scatter plots, contours, profiles, etc.).

Another problem associated with the use of different analytical methods is left censoring (i.e., data less than or equal to equipment detection limit). Porter *et al.* (1988) and Gilliom and Helsel (1986) provide a discussion on the implications of analyzing left censored data and alternative procedures for estimating summary statistics when the data are censored. It is generally not recommended to estimate summary statistics for left censored data.

B2.6 Use of Data

The results may be used to:

- establish spatial and temporal trends in the transport of pollutants discharged into ambient waters
- calculate nutrient budgets
- calibrate and verify hydrodynamic models
- develop water quality standards for receiving waters

In addition, these results may be used to monitor rates of recovery following environmental interventions and provide early warnings of potential impacts to the estuarine ecosystem.

Monitoring of pollutant levels in the water column is a widely-accepted means of measuring the condition of the aquatic habitat. However, the singular use of pollutant loading data to assess the condition of the water column or to guide the decision-making process in not recommended. Data acquired from monitoring water contaminant levels, in conjunction with the water's physical properties, may be used to assess the health risks to human populations. In addition, ecological risks to individuals, populations, and communities living in the water column may be evaluated. Monitoring of water column chemistry remains a powerful tool in the evaluation of spatial and temporal effects of anthropogenic and natural disturbances.

B2.7 Summary and Recommendations

Rationale

- Monitoring water quality parameters will provide information on ambient water conditions and the potential for transport and persistence of contaminants in the aqueous environment

Monitoring Design Considerations

- The objectives of the monitoring program must be clearly defined in order that:
  - the analytical methods show appropriate selectivity, specificity and sensitivity for the contaminants of concern
  - sampling and sample handling procedures are well-defined and consistent so as not to compromise the integrity and representativeness of the samples
  - sampling numbers and locations are appropriate to the level of information required

- Water column monitoring for hydrophobic chemicals is not recommended.

- Time of sampling can strongly influence data comparability.

Existing Analytical Methods

- Dissolved Oxygen
  - The Winkler (titrimetric) method is the first method of choice for the measurement of dissolved oxygen, unless the samples contain interferents such as sulfur compounds, chlorine, free iodine, color, turbidity, or biological flocs, or when continuous monitoring is planned. In those instances, a membrane electrode should be used

- Nutrients
  - nutrients such as ammonia nitrogen, total Kjeldahl nitrogen, nitrate-nitrite nitrogen, and phosphorus are typically determined by spectrophotometric measurements using a segmented continuous flow analyzer

  - the usual method for chlorophyll determination is fluorometric, however the choice of method is determined by the need to separate the different forms. HPLC is the most accurate method, however, it is also the most expensive

- Trace Metals
  - a combination of AA spectroscopy and ICP spectrometry is the recommended method for the detection of metals

- cold vapor AA spectroscopy is the recommended protocol for the detection of mercury

- Organics
  - for monitoring efforts where the most accurate quantitation is important, the isotope dilution GC/MS method is recommended for volatiles and semivolatiles. GC/ECD is the method of choice for pesticides and PCBs

QA/QC Considerations

- Calibration standards and blank, matrix spike, and replicate analyses are recommended quality control checks

- A report describing the objectives of the analytical effort, methods of sample collection, handling and preservation, details of the analytical method, problems encountered during the analytical process, any necessary modifications to the written procedures, and results of the QC analyses should be included with the quantitative data

Statistical Design Considerations

- The analyst typically estimates statistics for central tendency (e.g., mean, median) and variability (e.g., standard deviation, interquartile range) of grouped data. Uncertainty should be indicated by reporting estimates with confidence limits or percentiles

- For the purpose of estimating summary statistics, it is generally recommended not to use left censored data

- Prior to the collection of data, the statistical test (and significance level) used to analyze the data should be specified

Use of Data

- Monitor ambient levels of pollutants in the environment

- Establish spatial and temporal trends in the accumulation and transport of pollutants discharged into ambient waters

- Calibrate and verify mathematical models
- Calculate nutrient budgets
- Develop water quality standards for receiving waters
- Identify noncompliant discharges

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B3.0 Sediment Grain Size

B3.1 Rationale

The objective of monitoring sediment grain size composition is to detect and describe spatial and temporal changes of the benthic environment. The availability of sediment contaminants are often correlated with the grain size composition of the benthic medium; sediment contaminants are more easily adsorbed onto small grain sediment surfaces. Likewise, grain size information may explain the temporal and spatial variability in biological assemblages; changes in sediment grain size often affect an infaunal organism's ability to build tubes, capture food, and escape predation. These results may be used to monitor rates of recovery following environmental interventions, to evaluate the condition of benthic habitats, and to assist in providing early warnings of potential impacts to the estuarine ecosystem.

B3.2 Monitoring Design Considerations

Sediment Sampling Devices

Sampling crews have been given a wide latitude in how sediment samples are collected. In fact, the protocols required to collect an acceptable surficial sediment sample for subsequent measurement of physical characteristics have generally been neglected. However, because sample collection protocols influence all subsequent laboratory and data analysis, it is key that sediment samples be collected using acceptable and standardized techniques.

Collection of undisturbed sediment requires that the sampler:

- create a minimal pressure wave when descending
- form a leakproof seal when the sediment sample is taken
- prevent winnowing and excessive sample disturbance when ascending
- allow easy access to the sample surface in order that undisturbed subsamples may be taken

Penetration well below the desired sampling depth is preferred to prevent sample disturbance as the device closes. It is preferable to use a sampler that has the means of weight adjustment in order that penetration depths may be modified.

Numerous types of devices can be used to collect sediment samples. These can be divided into two general categories: grabs and box corers (McIntyre *et al.*, 1984; ASTM, 1991). Many of these devices sample the benthic habitat in a unique manner (Table B3-1). Accordingly, conducting comparisons among data collected using different devices is unadvisable.

TABLE B3-1. SUMMARY OF BOTTOM SAMPLING EQUIPMENT[a]

Device	Use	Advantages	Disadvantages
Fluorocarbon plastic or Glass Tube	Shallow wadeable waters or deep waters if SCUBA available. Soft or semi-consolidated deposits.	Preserves layering and permits historical study of sediment deposition. Rapid–samples immediately ready for laboratory shipment. Minimal risk of contamination.	Small sample size requires repetitive sampling.
Hand Corer with removable Fluorocarbon plastic or glass liners	Same as above except more consolidated sediments can be obtained.	Handles provide for greater ease of substrate penetration. Above advantages.	Careful handling necessary to prevent spillage. Requires removal of liners before repetitive sampling. Slight risk of metal contamination from barrel and core cutter.
Box corer	Same as above.	Collection of large undisturbed sample allowing for subsampling.	Hard to handle.
Gravity corers, that is, Phleger Corer	Semi-consolidated sediments.	Low risk of sample contamination. Maintains sediment integrity relatively well.	Careful handling necessary to avoid sediment spillage. Small sample, requires repetitive operation and removal of liners. Time consuming.
Young Grab (fluorocarbon plastic- or kynar-lined modified 0.1 m^2 van Veen)	Lakes and marine areas.	Eliminates metal contamination. Reduced pressure wave.	Expensive. Requires winch.
Ekman or Box Dredge	Soft to semi-soft sediments. Can be used from boat, bridge, or pier in waters of various depths.	Obtains a larger sample than coring tubes. Can be subsampled through box lid.	Possible incomplete jaw closure and sample loss. Possible shock wave which may disturb the fines. Metal construction may introduce contaminants. Possible loss of "fines" on retrieval.
PONAR Grab Sampler	Useful on sand, silt, or clay.	Most universal grab sampler. Adequate on most substrates. Large sample obtained intact, permitting subsampling.	Shock wave from descent may disturb "fines". Possible incomplete closure of jaws results in sample loss. Possible contamination from metal frame construction. Sample must be further prepared for analysis.

TABLE B3-1. SUMMARY OF BOTTOM SAMPLING EQUIPMENT[a] (continued)

Device	Use	Advantages	Disadvantages
BMH-53 Piston Corer	Waters of 4-6 ft deep when used with extension rod. Soft to semi-consolidated deposits.	Piston provides for greater sample retention.	Cores must be extruded on site to other containers–metal barrels introduce risk of metal contamination.
Van Veen	Useful on sand, silt, or clay.	Adequate on most substrates. Large sample obtained intact, permitting subsampling.	Shock wave from descent may disturb "fines". Possible incomplete closure of jaws results in sample loss. Possible contamination from metal frame construction. Sample must be further prepared for analysis.
BMH-60	Sampling moving waters from a fixed platform.	Streamlined configuration allows sampling where other devices could not achieve proper orientation.	Possible contamination from metal construction. Subsampling difficult. Not effective for sampling fine sediments.
Petersen Grab Sampler	Useful on most substrates.	Large sample; can penetrate most substrates.	Heavy, may require winch. No cover lid to permit subsampling. All other disadvantages of Ekman and Ponar.
Shipek Grab Sampler	Used primarily in marine waters and large inland lakes and reservoirs.	Sample bucket may be opened to permit subsampling. Retains fine grained sediments effectively.	Possible contamination from metal construction. Heavy, may require winch.
Orange-Peel Grab Smith-McIntyre Grab	Useful on most substrates.	Designed for sampling hard substrates.	Loss of fines. Heavy–may require winch. Possible metal contamination.
Scoops, Drag Buckets	Various environments depending on depth and substrate.	Inexpensive, easy to handle.	Loss of fines on retrieval through water column.

[a] Comments represent subjective evaluations.

SOURCE: American Society for Testing and Materials, 1991

Grab samplers – Grabs are capable of consistent sampling of bottom habitats. Depending on the size of the device, areas 0.02 to 0.5 m^2 and depths ranging from 5 to 15 cm may be sampled. Limitations of grab samplers include:

- variability among samples in penetration depth depending on sediment properties
- oblique angles of penetration which result in varying penetration depths within a sample
- the sample is inevitably folded, resulting in the inability to section the sample and the loss of information concerning the vertical structure in the sediments

However, the careful use of these devices will provide quantitative data. Grab samplers are the tools of choice for a number of estuarine and marine monitoring programs due to their ability to provide quantitative data at a relatively low cost (Fredette *et al.*, 1989; U.S. EPA, 1986-1991).

Core samplers – Box corers utilize a surrounding frame to ensure vertical entry; vertical sectioning of the sample is possible (U.S. EPA, 1987a). These devices are capable of maximum penetration depths of 15 cm and may collect volumes 5 to 10 times that of grab samplers. Limitations of box corers include:

- its large size and weight require the use of cranes or winches and a large vessel for deployment
- higher construction expenses
- lack of calibration studies to permit comparisons to grab samples

The Hessler-Sandia box corer uses dividers to section the core into subsections, facilitating subsampling of the core. Box corers are recognized as the tools of choice for maximum accuracy and precision when sampling soft bottom habitats.

Sediment Profiling Camera – The sediment profiling camera allows vertical *in situ* imaging of the water-sediment interface from which grain size determinations may be conducted. The sediment grain size determinations may be made at a maximum depth of 18 cm, however, penetration depth of the viewing prism may be limited due to the physical characteristics of the sediment (i.e., penetration depths are greater in silt than sand).

Distinction between finer silts and clays are not possible. Furthermore, it is recommended that quantitative calculations of sediment grain size from the image be verified by the results of laboratory analysis of collected sediment samples.

The singular use of the sediment profiling camera is not recommended. However, the sediment profile camera proves to be effective as a reconnaissance tool. Delineation of habitats with similar physical characteristics may aid in the selection of appropriate sampling stations.

Recommendations – Collection of sediments and benthic organisms should be done concurrently in order to mitigate the costs of field sampling and to permit sound correlation, regression, and multivariate analyses (see Benthic Community Structure). Therefore, it is recommended that the sampling device also be suitable for benthic sampling. Grab and core sampling devices permit adequate sampling of both sediment and benthic infaunal communities with the one sampling device.

Sample Depth

It is recommended that the upper 2 cm of the sediment column be examined to characterize surficial sediments. Although the 2 cm specification is arbitrary, it will ensure that:

- relatively recent sediments are sampled
- adequate volumes of sediments are readily obtained for laboratory analyses
- data from different studies may be compared

Sampling of depths other than 2 cm or vertical stratification of deeper sediment cores may be appropriate, depending upon the objectives of the monitoring program and the rate of sediment accumulation. For example, if information concerning only the most recent sedimentation events is required, examination of the upper 1 cm may be appropriate. Stratification of deeper cores will provide historical data of sediment grain size and depositional events. If the potential for bioaccumulation of contaminants in infaunal organisms is a concern, sampling to the depth of the anoxic layer is desirable. Comparison of data from studies analyzing different sediment depths is not advised. Penetration well below the desired sampling depth is preferred to prevent sample disturbance as the device closes.

Selection of Time of Sampling

Sediment grain size compositions are often temporally stable, although some slight seasonal variability may be present. Changes are usually associated with seasonal patterns of benthic turbulent mixing and sediment transport phenomena. The frequency of sampling should be related to the expected rate of change in grain size compositions. A consistent sampling period is recommended in order that spatial and temporal comparisons may be conducted.

If seasonal variations are exhibited, it is recommended that direct comparisons between samples collected during different seasons be avoided. Studies investigating interannual variation in the percent composition of grain sizes should be conducted during the same season (preferably the same month) each year. Furthermore, it is recommended that grain size be sampled when contaminant concentrations are expected to be at their highest level in order to evaluate worst-case scenarios.

B3.3 Existing Analytical Methods

Grain Size

Sediment grain size may be expressed in either mm or ϕ (phi) units. These scales are related according to the equation: $\phi = -\log_2$ (mm). Data should be converted to phi units before calculation of grain size parameters. Sediments are broadly classified into three size classes: silts and clays are less than 0.064 mm (4 ϕ) in diameter, sands range from 0.064 mm (4 ϕ) to 1 mm (0 ϕ) in diameter, and gravels are larger than 1 mm (Shepard, 1954; Folk, 1974). Grain size is normally reported as the mean (M_z), although the median grain size ($M_{d\phi}$) is sometimes used. Sorting is a measure of the spread of the grain size distribution. Buller and McManus (1979) provide a good review of the methodological and statistical analysis of sediment samples.

True vs. Apparent Particle Size

Particle size determination can either include or exclude organic material. If organic material is removed prior to analysis, the "true" particle size distribution is determined. If organic material is included in the analysis, the "apparent" particle size distribution is ascertained. Most organic material is in the silt/clay size range and can be removed from the sediment either by acid washing or ashing. If organic material is left in the sediments, it will tend to bias the results toward a smaller mean size. Because true and apparent distributions differ, detailed comparisons between samples analyzed by these different methods are questionable. It is therefore recommended that measures of sediment grain size be examined using only one of these methods. A standardized

grain size analysis will allow all comparisons between samples within each study and among different studies.

Sieving

Sieving separates the sediment sample into size fractions. Wet sieving separates the sample into two fractions: grain size greater than 62.5 μm (i.e., sand and gravel) and less than 62.5 μm (i.e., silt and clay). Aggregates should be gently broken and wet sieving should continue until only clear water passes through the sieve.

The gravel-sand fraction can be further divided by dry sieving through a graded series of screens (U.S. EPA, 1986-1991). The sample should be shaken on the mesh for a pre-determined, standard amount of time. The last sieve should have a mesh size of 62.5 μm (4 phi) and any material passing through this sieve should be added to the silt-clay fraction of that sample. The silt-clay fraction can be subdivided using a pipet technique (U.S. EPA, 1986-1991). This technique depends upon differential settling rates of different sized particles. Withdrawal time for pipet analysis, as a function of particle size and water temperature, is given in Table B3-2. The total weight of each phi-size interval must then be calculated (U.S. EPA, 1986-1991).

An alternate method for the determination of grain size for the sand fraction of the sediment is the use of a settling tube. This technique is based on Stoke's Law which describes the sinking rate of a particle relative to its diameter. This technique requires a much smaller sample size (0.5-1.0 gm) as compared to 100 gm required for dry sieving. Furthermore, settling tube analysis is relatively rapid, and automated settling tubes which input the data directly to a personal computer are available.

It is key that the analytical techniques and the desired number of subfractions be specified and standardized to allow for comparisons between samples.

B3.4 QA/QC Considerations

It is critical that each sample be homogenized thoroughly in the laboratory before a subsample is taken for analysis. Laboratory homogenization should be conducted even if samples were homogenized in the field as sediments will differentially sort themselves during transport and handling. In addition, after dry-sieving a sample all material must be removed from the sieve.

The total amount of fine-grained material used for pipet analysis should be 5-25 grams. If more material is used, particles may interfere with each other during settling and the possibility of flocculation may be enhanced. If less material is used, the experimental error in weighing becomes large relative to the sample size. Once the

TABLE B3-2. SEDIMENT GRAIN SIZE: WITHDRAWAL TIMES FOR PIPET ANALYSIS AS A FUNCTION OF PARTICLE SIZE AND WATER TEMPERATURE[a,b]

Diameter Finer than (phi)[c]	Diameter Finer than (μm)	Withdrawal Depth (cm)	Elapsed Time for Withdrawal of Sample in Hours (h), Minutes (m), and Seconds (s)						
			18°C	19°C	20°C	21°C	22°C	23°C	24°C
4.0	62.5	20	20s	20s	20s	20s	20s	20s	20s
5.0	31.2	10	2m0s	1m57s	1m54s	1m51s	1m49s	1m46s	1m44s
6.0	15.6	10	8m0s	7m48s	7m36s	7m25s	7m15s	7m5s	6m55s
7.0	7.8	10	31m59s	31m11s	30m26s	29m41s	28m59s	298m18s	27m39s
8.0[d]	3.9	10	2h8m	2h5m	2h2m	1h59m	1h56m	1h53m	1h51m
9.0	1.95	10	8h32m	8h18m	8h6m	7h56m	7h44m	7h32m	7h22m
10.0	0.98	10	34h6m	33h16m	32h28m	31h40m	30h56m	30h12m	29h30m

[a] Modified from Plumb (1981).
[b] It is critical that temperature be held constant during the pipet analysis.
[c] $\phi = -\log_2$ [particle diameter (mm)].
[d] Breakpoint between silt and clay.

pipet analysis begins, the settling cylinders must not be disturbed, as this will alter particle settling velocities.

It is recommended that triplicate analyses be conducted on one of every 20 samples, or on one sample per batch if less than 20 samples are analyzed. It is recommended that the analytical balance, drying oven, and temperature bath be inspected daily and calibrated at least once per week.

B3.5 Statistical Design Considerations

Statistical strategies may mitigate the high costs of collecting sufficient quantities of sediment. See also Statistical Design Considerations: Composite Sampling, Power Analysis, and Power Cost Analysis (Section B.3).

B3.6 Use of Data

Sediment grain size provides evidence essential in the evaluation of spatial and temporal effects of anthropogenic and natural disturbances.

Grain size information may explain the temporal and spatial variability in biological assemblages. Because many infaunal organisms are sensitive to changes in grain size (e.g., affects their ability to burrow, to construct tubes), changes in benthic community structure may be explained by simply examining grain size characteristics.

The detection of a change in sediment grain size composition may provide an early warning of a potential threat to sensitive resources, such as a threat to commercial clam beds. For many commercially and recreationally important clams, the ability to quickly burrow, and to escape predation, is dependent upon the sediment grain size composition of the bottom. Thus, changes of sediment grain size may be a good indicator of impending loss of important resources.

However, the singular use of sediment grain size to assess the condition of the benthic habitat or to guide the decision-making process is not recommended. The data garnered from monitoring of the sediment's physical characteristics should be used in conjunction with that collected from chemical and biological monitoring. Subsequent analyses of health risks to human populations, as well as ecological risks to benthic individuals, populations, and communities may be evaluated.

B3.7 Summary and Recommendations

Rationale

- The objective of monitoring the sediment's physical characteristics is to detect and describe spatial and temporal changes in these characteristics.

- Results may be used to: monitor rates of recovery following environmental interventions; assist in interpreting the results of physical and biological monitoring programs; evaluate the condition of benthic habitats, and; provide early warnings of potential impacts to the estuarine ecosystem.

Monitoring Design Considerations

- It is recommended that consistent types of sampling gear, sampling location, and timing of sample collection be implemented to allow for comparisons among studies

- Collection of undisturbed sediment requires that the sampler:
  - create a minimal pressure wave when descending
  - form a leakproof seal when the sediment sample is taken
  - prevent winnowing and excessive sample disturbance when ascending
  - allow easy access to the sample surface in order that undisturbed subsamples may be taken

- In general, analysis of the upper 2 cm will allow examination of recent depositional events; however, site-specific conditions and program objectives should be considered when a sampling depth is selected

- Penetration well below the desired sampling depth is preferred to prevent sample disturbance as the device closes.

Existing Analytical Methods

- Because true and apparent distributions differ, detailed comparisons between samples analyzed by these different methods are questionable

- It is therefore desirable that all samples within each study and among different studies be analyzed using the same method (i.e., either including or excluding organic material)

QA/QC Considerations

- Laboratory homogenization should be conducted even if samples were homogenized in the field

- It is recommended that the analytical balance, drying oven, and temperature bath be inspected daily and calibrated at least once per week

Statistical Design Considerations

- Compositing sediment samples consists of mixing two or more field replicates or field samples collected at a particular location and time
- Space- (combining composites from several locations) and/or time- (combining several composites over time from one location) bulking strategies should be used judiciously since information concerning spatial and temporal heterogeneity may be lost
- Power analyses have shown that for a fixed level of sampling effort, a monitoring program's efficiency is generally increased by collecting more replicates at fewer locations

Use of Data

- Grain size information may explain the temporal and spatial variability in biological assemblages
- The singular use of sediment grain size to assess the condition of the benthic habitat or to guide the decision-making process in not recommended; data garnered from monitoring of the sediment's physical characteristics should be used in conjunction with that collected from chemical and biological monitoring

B3.8 Literature Cited and References

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B4.0 Sediment Chemistry

B4.1 Rationale

The sediments represent the ultimate repository for many chemical contaminants in the estuarine environment (U.S. EPA, 1992). Sediments also provide habitats for many aquatic organisms. The objective of monitoring bulk sediment chemistry is to detect and describe spatial and temporal changes of these sediment pollutants. The results may be used to evaluate the condition of benthic habitats, to provide early warnings of potential impacts to the estuarine ecosystem, and to monitor rates of ecosystem recovery following environmental interventions.

Monitoring of pollutant levels in sediments is a widely accepted means of measuring the condition of the benthic habitat and is a powerful tool for the evaluation of spatial and temporal effects of anthropogenic and natural disturbances. The singular use of sediment pollutant loading data to assess the condition of the benthic habitat or to guide the decision-making process is not recommended since other factors, such as water quality and sediment grain size, can also affect habitat quality.

B4.2 Monitoring Design Considerations

Sediment Sampling Devices

The protocols required to collect an acceptable surficial sediment sample for subsequent measurement of chemical variables have generally been neglected. In fact, sampling crews have been given wide latitude in determining how samples are collected. However, because sample collection protocols influence all subsequent laboratory and data analysis, it is key that sediment samples be collected using acceptable and standardized techniques.

Collection of undisturbed sediment requires that the sampler:

- create a minimal pressure wave when descending
- form a leakproof seal when the sediment sample is taken
- prevent winnowing and excessive sample disturbance when ascending
- allow easy access to the sample surface in order that undisturbed subsamples may be taken (ASTM, 1991)

Penetration well below the desired sampling depth is preferred to prevent sample disturbance as the device closes. It is optimal to use a sampler that has the means of weight adjustment in order that penetration depths may be modified.

Several types of devices can be used to collect sediment samples: dredges, grabs, and box corers (McIntyre *et al.*, 1984). Each of these devices sample the benthic habitat in a unique manner (Table B4-1). Accordingly, conducting comparisons among data collected using different devices is inadvisable.

Grab samplers – Grabs are capable of consistent sampling of bottom habitats. Depending on the size of the device, areas of 0.02 to 0.5 m^2 and depths ranging from 5 to 15 cm may be sampled. Limitations of grab samplers include:

- variability among samples in penetration depth depending on sediment properties
- oblique angles of penetration which result in varying penetration depths within a sample
- the sample is inevitably folded resulting in the inability to section the sample and the loss of information concerning the vertical structure in the sediments (U.S. EPA, 1991b)

However, quantitative data can be provided by the careful use of these devices. Grab samplers are the tools of choice for a number of estuarine and marine monitoring programs due to their ability to provide reliable quantitative data at a relatively low cost (Fredette *et al.*, 1989; U.S. EPA, 1986-1991).

Core samplers – Box corers utilize a surrounding frame to ensure vertical entry; vertical sectioning of the sample is possible (U.S. EPA, 1986-1991). These devices are capable of maximum penetration depths of 15 cm and may collect volumes 5 to 10 times that of grab samplers. Limitations of box corers include:

- its large size and weight require the use of cranes or winches and a large vessel for deployment
- higher construction expenses
- lack of calibration studies to permit comparisons to grab samples

The Hessler-Sandia box corer uses dividers to section the core into subsections, facilitating subsampling of the core. Box corers are recognized as the tools of choice for maximum accuracy and precision when sampling soft bottom habitats.

TABLE B4-1. SUMMARY OF BOTTOM SAMPLING EQUIPMENT[a]

Device	Use	Advantages	Disadvantages
Fluorocarbon plastic or Glass Tube	Shallow wadeable waters or deep waters if SCUBA available. Soft or semi-consolidated deposits.	Preserves layering and permits historical study of sediment deposition. Rapid–samples immediately ready for laboratory shipment. Minimal risk of contamination.	Small sample size requires repetitive sampling.
Hand Corer with removable Fluorocarbon plastic or glass liners	Same as above except more consolidated sediments can be obtained.	Handles provide for greater ease of substrate penetration. Above advantages.	Careful handling necessary to prevent spillage. Requires removal of liners before repetitive sampling. Slight risk of metal contamination from barrel and core cutter.
Box corer	Same as above.	Collection of large undisturbed sample allowing for subsampling.	Hard to handle.
Gravity corers, that is, Phleger Corer	Semi-consolidated sediments.	Low risk of undisturbed sample contamination. Maintains sediment integrity relatively well.	Careful handling necessary to avoid sediment spillage. Small sample, requires repetitive operation and removal of liners. Time consuming.
Young Grab (fluorocarbon plastic- or kynar-lined modified 0.1 m^2 van Veen)	Lakes and marine areas.	Eliminates metal contamination. Reduced pressure wave.	Expensive. Requires winch.
Ekman or Box Dredge	Soft to semi-soft sediments. Can be used from boat, bridge, or pier in waters of various depths.	Obtains a larger sample than coring tubes. Can be subsampled through box lid.	Possible incomplete jaw closure and sample loss. Possible shock wave which may disturb the fines. Metal construction may introduce contaminants. Possible loss of "fines" on retrieval.
PONAR Grab Sampler	Useful on sand, silt, or clay.	Most universal grab sampler. Adequate on most substrates. Large sample obtained intact, permitting subsampling.	Shock wave from descent may disturb "fines". Possible incomplete closure of jaws results in sample loss. Possible contamination from metal frame construction. Sample must be further prepared for analysis.

TABLE B4-1. SUMMARY OF BOTTOM SAMPLING EQUIPMENT[a] (continued)

Device	Use	Advantages	Disadvantages
BMH-53 Piston Corer	Waters of 4-6 ft deep when used with extension rod. Soft to semi-consolidated deposits.	Piston provides for greater sample retention.	Cores must be extruded on site to other containers–metal barrels introduce risk of metal contamination.
Van Veen	Useful on sand, silt, or clay.	Adequate on most substrates. Large sample obtained intact, permitting subsampling.	Shock wave from descent may disturb "fines". Possible incomplete closure of jaws results in sample loss. Possible contamination from metal frame construction. Sample must be further prepared for analysis.
BMH-60	Sampling moving waters from a fixed platform.	Streamlined configuration allows sampling where other devices could not achieve proper orientation.	Possible contamination from metal construction. Subsampling difficult. Not effective for sampling fine sediments.
Petersen Grab Sampler	Useful on most substrates.	Large sample; can penetrate most substrates.	Heavy, may require winch. No cover lid to permit subsampling. All other disadvantages of Ekman and Ponar.
Shipek Grab Sampler	Used primarily in marine waters and large inland lakes and reservoirs.	Sample bucket may be opened to permit subsampling. Retains fine grained sediments effectively.	Possible contamination from metal construction. Heavy, may require winch.
Orange-Peel Grab Smith-McIntyre Grab	Useful on most substrates.	Designed for sampling hard substrates.	Loss of fines. Heavy–may require winch. Possible metal contamination.
Scoops, Drag Buckets	Various environments depending on depth and substrate.	Inexpensive, easy to handle.	Loss of fines on retrieval through water column.

[a] Comments represent subjective evaluations.

SOURCE: American Society for Testing and Materials, 1991

Recommendations – Collection of sediments and benthic organisms should be done concurrently in order to mitigate the costs of field sampling and to permit sound correlation, regression, and multivariate analyses (see Benthic Community Structure). Therefore, it is recommended that the sampling device also be suitable for benthic sampling. Grab and core sampling devices permit adequate sampling of both sediment and benthic infaunal communities with the one sampling device.

Sample Depth

It is recommended that the upper 2 cm of the sediment column be examined to characterize surficial sediments. Although the 2 cm specification is arbitrary, it will ensure that:

- relatively recent sediments are sampled
- adequate volumes of sediments are readily obtained for laboratory analyses
- data from different studies may be compared

Sampling of depths other than 2 cm or vertical stratification of deeper sediment cores may be appropriate, depending upon the objectives of the monitoring program and the rate of sediment accumulation. For example, if information concerning only the most recent sediment contamination is required, examination of the upper 1 cm may be appropriate. Stratification of deeper cores will provide historical data of sediment contaminant levels and depositional events. If the potential for bioaccumulation of contaminants in infaunal organisms is a concern, sampling to the depth of the anoxic layer is desirable. Comparison of data from studies analyzing different sediment depths is not advised. Penetration well below the desired sampling depth is preferred to prevent sample disturbance as the device closes.

Total Organic Carbon and Acid Volatile Sulfides Normalization

Total organic carbon (TOC) and acid volatile sulfides (AVS) are considered by many to be the most important parameters in defining organic and metal concentrations in sediments. Toxic hydrophobic contaminant concentrations have been found to be related to the TOC content of the sediment (Karickhoff *et al.*, 1979). Also, the concentrations of some divalent toxic metals have been found to be related to the AVS concentration of the sediment (DiToro *et al.*, in press). The AVS pool is available to bind metals, thereby reducing their bioavailability.

Total Organic Carbon (TOC) normalizations of organic contaminant concentrations have been used to provide estimates of bioavailability. These normalizations appear to account for some of the variability found in bioaccumulation rates and biological community structure. TOC/lipid normalized accumulation factors (AF) have also been used to predict tissue residue concentrations (Ferraro *et al.*, 1990; Lake *et al.*, 1987).

Normalization based on AVS concentrations is considered a potentially useful tool for assessing the bioavailability of certain divalent metals (e.g., Cd and Ni). However, only a few laboratories can perform these analyses at present and the procedure has not undergone any round robin verification. In addition, sampling for AVS is very subject to error as contact with air can greatly effect results. Although AVS normalizations represent a promising tool for evaluation of sediment metals data, general use in estuarine monitoring programs may not yet be practical.

These normalizations assume the following:

- organic contaminants partition predominantly to sediment organic carbon; metal contaminants partition predominantly to sediment AVS
- rapid steady-state kinetics of contaminants

Development of standardized methods for measuring sediment TOC and AVS in the laboratory are required before normalized contaminant concentrations may be compared among studies.

The decision to normalize for TOC and AVS will depend upon monitoring program objectives. For example, if the objective is to identify the "footprint" of a discharge, normalization may not be appropriate. However, if the monitoring objective is to identify "hot spots" (i.e., those areas where bioavailable contaminants represent a risk to human and ecological health), normalization may be justified.

Selection of Sampling Period

Sediment contaminant levels typically display temporal patterns due to seasonal variability. These patterns are usually a result of seasonal changes in benthic turbulent mixing and sediment transport phenomena. The frequency of sampling should be related to the expected rate of change in sediment contaminant concentrations. A spatially and temporally consistent sampling design (i.e., time of sampling event and sampling locations do not change over time) is recommended in order that comparisons between areas over time may be conducted.

If seasonal variations are exhibited, it is recommended that direct comparisons between samples collected during different seasons be avoided. Studies investigating interannual variation in the concentrations of sediment contaminants should be conducted during the same season (preferably the same month) each year. Furthermore, it is recommended that sediments be sampled when contaminant concentrations are expected to be at their highest level in order to evaluate worst-case scenarios.

B4.3 Existing Analytical Methods

Questions to be considered during the choice of appropriate analytical methods include the parameters of interest, desired detection limits, sample size requirements or restrictions, methods of preservation, technical and practical holding times, and matrix interferences. It will frequently be necessary to use methods other than those currently approved by the EPA to achieve a desired sensitivity in an estuarine environment. However, alternative methods should be considered only when they have been demonstrated to provide a level of precision and accuracy equivalent to or exceeding that of the EPA approved method. Any proposed changes in analytical methods should be reviewed by analysts with experience in estuarine and coastal waters for applicability to that specific estuary.

Several U.S. EPA documents (e.g., 1986a and 1986-1991) discuss the common analytical problems encountered during analyses of sediment samples in monitoring programs. Those estuary programs that have established monitoring programs may provide an excellent source of information about analytical methods being developed for estuarine systems.

Chemical Residue Analyses

Several factors determine achievable detection limits for a specific contaminant, regardless of analytical procedure – a list of analytical procedures and U.S. EPA method numbers is given in Table B4-2. These factors include:

- sample size; 50-100 g (wet weight) with a minimum final dilution volume of 0.5 ml is considered adequate (U.S. EPA, 1986-1991)
- presence of interfering substances
- range of pollutants to be analyzed – the optimal method for one target pollutant may not be optimal for others

- level of confirmation – qualitative (e.g., presence or absence) or quantitative (e.g., residue concentrations) analyses

- level of pollutant found in the field and in analytical blanks

Selection of appropriate methods will be based on a trade-off between full-scan analyses, which are economical but cannot provide maximum sensitivity for some compounds, and alternate methods that are more sensitive for specific compounds but can result in higher analytical costs.

Metals and Metalloids – Digestion methods for sediment samples are reviewed by Plumb (1981). The U.S. EPA Contract Laboratory Program (CLP) requires the use of HNO_3:H_2O_2 (U.S. EPA, 1991a).

Trace element analyses by ICP (U.S. EPA method 6010) allows several elements to be measured simultaneously. Detection limits of ICP for most metals are generally comparable to those achieved by GFAAS; however, detection limits for some metals (arsenic, selenium, and mercury) are significantly lower using AAS.

TABLE B4-2. LIST OF EXISTING ANALYTICAL TECHNIQUES (U.S. EPA, 1986a)

METALS/METALLOIDS	
• Atomic Absorption Spectrophotometry (AAS)	U.S. EPA Method 7000 series
- flame	
- graphite furnace (GFAA)	
- cold vapor	U.S. EPA Method 7470
- gaseous hydride (HYDAAS)	U.S. EPA Methods 7060 and 7740
• Inductively Coupled Plasma Emission Spectrometry (ICP)	U.S. EPA Method 6010
ORGANICS	
• Gas Chromatography (GC)	
- with electron capture detection (GC/ECD)	U.S. EPA Method 8080
- with mass spectrometry (GC/MS)	U.S. EPA Methods 8240 and 8270

ICP – Al, Sb, As, Ba, Be, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, Mg, Mn, Mo, Ni, K, Se, Sn, Ag, Na, Tl, V, and Zn

AAS – Al, Sb, As, Ba, Be, Cd, Ca, Cr, Co, Cu, Fe, Pb, Mg, Mn, Hg, Mo, Ni, K, Se, Ag, Na, Tl, Sn, V, and Zn

The combination of atomic absorption spectrophotometry (AAS, U.S. EPA Methods 7000 series) and inductively coupled plasma emission spectrometry (ICP) is the recommended analytical method for detection of metals and metalloids since no technique is best for all elements (U.S. EPA, 1986a). Cold vapor AAS (U.S. EPA method 7470) analysis is the only recommended technique for mercury (U.S. EPA, 1986a).

Graphite furnace AAS (GFAAS) is more sensitive (i.e., lower detection limits) than flame AAS, but is more sensitive to matrix interference and spectral influences – GFAAS requires particular caution with regard to laboratory contamination and consequently, GFAAS requires more skilled laboratory technicians. Both AAS methods require that the concentration of each element be determined by a separate analysis, making the testing of a large number of contaminant metals both labor-intensive and relatively expensive compared to ICP. However, AAS methods may be cost-effective when testing for a few metals over a large number of samples.

Semi-Volatile Organic Compounds – Analysis of semi-volatile organic compounds involves a solvent extraction of the sample, cleanup of the characteristically complex extract, GC analysis, and quantification (U.S. EPA, 1986a, 1987b). There are two GC/MS options for detecting extractable organic compounds: internal standard technique and isotope dilution. The isotope dilution technique, which requires spiking the sample with a mixture of stable isotope labeled analogs of the analytes, is recommended because reliable recovery corrections can be made for each analyte with a labeled analog or a chemically similar analog (U.S. EPA, 1986a). The isotope dilution method is more expensive and less widely employed than the internal standard technique, which is the current method of choice in the Contract Laboratory Program (CLP).

Mass spectrometry provides positive compound identification by comparison of both retention time and spectral patterns with standard compounds. However, this technique lacks the sensitivity to detect levels of most trace contaminants in estuarine systems. Alternative methods providing greater sensitivity are available, but will be compound specific and, therefore, more expensive.

The identification of pesticides and PCBs can be made by GC/ECD analysis (U.S. EPA Method 8080). GC/ECD provides greater sensitivity relative to GC/MS, however GC/ECD does not provide positive compound identification. Confirmation of pesticides and PCBs on an alternative GC/ECD or preferably by GC/MS, when sufficient concentrations occur, is recommended for reliable results (U.S. EPA, 1986a). The detection and concentration of PCBs can be more accurately determined using comparison mixtures other than the standard industrial Aroclor mixtures. Evaluations of

PCB assemblages in environmental samples by quantification as Aroclors or total PCBs are of limited accuracy due to environmental degradation and differential affinities of PCB congeners for different environmental compartments. A more meaningful evaluation can be accomplished by quantification by PCB isomer groupings (dependent on the number of chlorine atoms per molecules). This has the advantage of indicating the relative concentrations of the groups containing the most toxic and bioaccumulating congeners (McFarland *et al.*, 1986). An alternative method of analysis is to test for the individual PCB congeners. Using congener-specific methods instead of Aroclor standard mixtures provides more accurate identification and quantification and eliminates the necessity of subjective decisions on the part of the analyst (Eganhouse, 1990). However, beside being more expensive and time consuming, the compatibility of these enhanced detection methods with the standard Aroclor method is an important consideration for monitoring programs with existing historical data. All other organic compound groups are recommended for analysis by GC/MS (U.S. EPA, 1986a).

Volatile Organic Compounds – Analysis of volatile organic compounds involves a solvent extraction of the sample, cleanup of the complex extract, GC analysis, and quantification (U.S. EPA, 1986a and 1987a). The purge and trap GC/MS technique is employed for detecting volatile organic compounds in sediments. GC/ECD may be used to achieve lower detection limits, although identification of the compounds may not be as accurate.

A successful variation for detection of volatile organic residues in sediments involves a device that vaporizes volatile organic compounds from the sediment sample under vacuum and then condenses the volatiles in a super-cooled trap (Hiatt, 1981). The trap is then transferred to a purge and trap device where it is treated as a water sample. The isotope dilution method is recommended as it provides reliable recovery data for each analyte (U.S. EPA, 1986a). However, this technique costs additional analytical time and expense.

B4.4 QA/QC Considerations

Sample Collection

Sampling Gear – The primary criterion for an adequate sampler is that it consistently collect undisturbed and uncontaminated samples. Sediment sampling devices should be inspected for wear and tear leading to sample leakage upon ascent. It is prudent to have a backup sampler on board the survey vessel in case the primary sampler is lost, damaged, or found to be unsuitable during the cruise.

In the field, sources of contamination include sampling gear, lubricants and oils, engine exhaust, airborne dust, and ice used for cooling samples. Samples designated for chemical analyses require all sampling equipment – e.g., siphon hoses, scoops, containers – be made of noncontaminating material and be cleaned appropriately prior to use. Potential airborne

contamination – e.g., stack gases, cigarette smoke – should be avoided. Furthermore, samples and sampling containers should not be touched with ungloved fingers.

Sample Condition – Benthic samples should satisfy the following sample acceptability criteria (U.S. EPA, 1986-1991):

- sampler is not over-filled with sample so that the sediment surface is pressed against the top of the sampler
- overlying water is present, indicating minimal leakage
- the overlying water is not excessively turbid indicating minimal sample disturbance
- the sediment surface is relatively flat indicating minimal disturbance or winnowing (Figure B4-1)
- the desired penetration depth is achieved

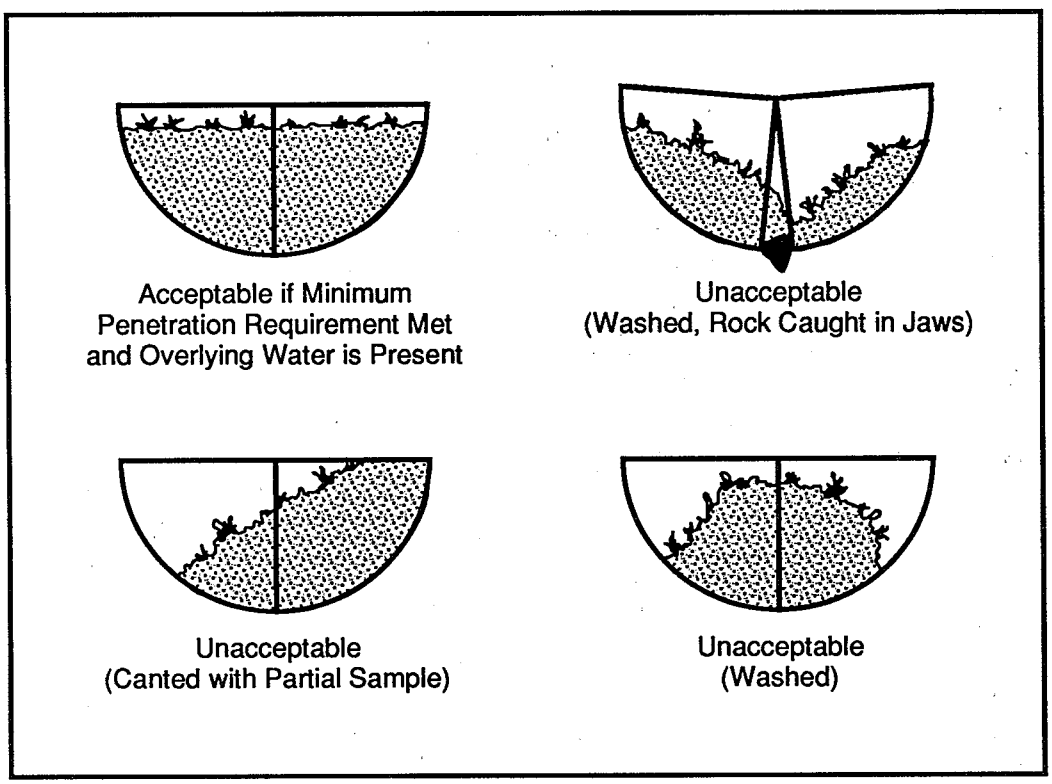

Figure B4-1. Examples of acceptable and unacceptable samples (U.S. EPA, 1986-1991).

If the sample does not meet all the criteria, the sample should be rejected.

Sample Handling

For analyses of metals, samples should be frozen and kept at -20°C (U.S. EPA, 1987a). Although specific holding times have not been recommended by U.S. EPA, a maximum of 6 months (8 days for mercury; ASTM 1991) would be consistent with holding times for water samples (Tables B4-3 and B4-4).

TABLE B4-3.
SUMMARY OF SAMPLE COLLECTION AND PREPARATION QA/QC REQUIREMENTS FOR ORGANIC COMPOUNDS

Variable	Sample Size[a]	Container[b]	Preservation	Maximum Holding Time
Semivolatiles	50-100 g	G	Freeze	6 months[c]
Volatiles	40 ml	G[d]	Cool, 4°C[e]	14 days
TOC	100 ml	G	Phosphoric acid, pH<2, refrigerate	28 days
TVS	100-500 ml	-	under an inert atmosphere	none recommended

[a] Recommended field sample sizes for one laboratory analysis. If additional laboratory analyses are required (i.e., lab replicates) the field sample size should be adjusted accordingly.

[b] G = Glass.

[c] This is a suggested holding time. No U.S. EPA criteria exist for the preservation of this variable.

[d] No headspace or airpockets should remain.

[e] Freezing these samples will likely cause breakage of the sample container, because no airspace for expansion is provided.

Table B4-4.
SAMPLING CONTAINERS, PRESERVATION REQUIREMENTS, AND HOLDING TIMES FOR SEDIMENT SAMPLES

Contaminant	Container[a]	Preservation	Holding Time
Acidity	P, G	Cool, 4°C	14 days
Alkalinity	P, G	Cool, 4°C	14 days
Ammonia	P, G	Cool, 4°C	28 days
Sulfate	P, G	Cool, 4°C	28 days
Sulfide	P, G	Cool, 4°C	28 days
Sulfite	P, G	Cool, 4°C	48 hours
Nitrate	P, G	Cool, 4°C	48 hours
Nitrate-Nitrite	P, G	Cool, 4°C	28 days
Nitrite	P, G	Cool, 4°C	48 hours
Oil and Grease	G	Cool, 4°C	28 days
Organic Carbon	P, G	Cool, 4°C	28 days
Metals			
Chromium VI	P, G	Cool, 4°C	40 hours
Mercury	P, G		8 days
Metals, except above	P, G		6 months
Organic Compounds			
Extractables (including phthalates, nitrosamines, organochlorine pesticides, PCB's, nitroaromatics, isophorone, Polynuclear aromatic hydrocarbons, haloethers, chlorinated hydrocarbons and TCDD)	G, teflon-lined cap	Cool, 4°C	7 days, (until extraction) 30 days (after extraction)
Extractables (phenols)	G, teflon-lined cap	Cool, 4°C	7 days (until extraction) 30 days (after extraction)
Purgeables (halocarbons and aromatics)	G, teflon-lined septum	Cool, 4°C	14 days
Purgeables (acrolein and acrylonitrile)	G, teflon-lined septum	Cool, 4°C	3 days
Orthophosphate	P, G	Cool, 4°C	48 hours
Pesticides	G, teflon-lined cap	Cool, 4°C	7 days (until extraction) 30 days (after extraction)
Phenols	P, G	Cool, 4 °C	28 days
Phosphorus (elemental)	G	Cool, 4°C	48 hours
Phosphorus, total	P, G	Cool, 4°C	28 days
Chlorinated organic compounds	G, teflon-lined cap	Cool, 4°C	7 days (until extraction) 30 days (after extraction)

[a] Polyethylene (P) or Glass (G)

SOURCE: American Society for Testing and Materials, 1991

For analyses of volatile compounds, samples should be stored in the dark at 4°C (U.S. EPA, 1987a). Analyses of volatile compounds should be performed within 14 days of collection as recommended by U.S. EPA (1990). If analyses of semivolatile compounds will not be performed within the recommended 7-day holding time, freezing of the samples at -20°C is advised. Holding times for frozen samples has not been established by the EPA. A general guideline of a maximum of 6 months would be consistent with water sample guidelines (U.S. EPA, 1987a).

Samples for determination of TOC or AVS should be analyzed as soon as possible. If not analyzed immediately, TOC samples should be refrigerated and their pH brought below 2 by addition of phosphoric acid. Acidification is recommended only when inorganic carbon is below detection limits (APHA, 1990). Acid Volatile Sulfides should be stored in airtight containers under an inert atmosphere and analyzed as soon as possible.

Resectioning sediment samples for chemical analyses should only be conducted with noncontaminating tools under "clean room" conditions.

Laboratory Analyses

Laboratory performance as measured by method QA/QC protocols should be used to evaluate and select appropriate analytical methods. Changes to laboratory protocols should only be considered if new protocols meet established performance criteria.

QA/QC reports should describe the results of quantitative QA/QC analyses, as well as other elements critical to the laboratory analyses of water, sediments, and tissues to ensure proper interpretation of the results. It is recommended that these reports be recorded and stored in a database for future reference.

Field QA/QC Checks – Travel (trip) blanks can indicate whether contamination was introduced by reagents in the field or introduced during shipping of samples. Rinsate blanks are designed to verify the absence of contamination that can be carried over from one sample to another due to inadequate cleaning of field equipment.

Field splits, treated and identified as separate samples, may be sent to the same laboratory for analysis or one sample may be sent to a "reference" laboratory for comparison. In addition, standard reference material may also be placed in a sample container at the time of collection and sent "blind" to the laboratory.

Instrument QA/QC Checks – Calibration standards should be analyzed at the beginning of sample analysis, and should be verified at the end of each 12-hr shift during which analyses are performed (U.S. EPA, 1987a). The concentrations of calibration standards should bracket the expected sample concentrations, otherwise sample dilutions or sample handling modifications (i.e., reduced sample size) will be required.

Method QA/QC Checks – Analysis of method blanks should be conducted to demonstrate the absence of contamination from sampling or sample handling in the laboratory. At least one method blank must be included with each batch of samples and should constitute at least 5% of all samples analyzed.

Spike recovery analyses are required to assess method performance for the particular sample matrix. Spike recoveries serve as an indication of analytical accuracy, whereas analysis of standard reference materials (SRM) measure extraction efficiency. Recommended control limits include 75-125 percent recovery for spikes, and 80-120 percent recovery for SRM.

Replicates must be analyzed to monitor the precision of laboratory analyses. A minimum of 5% of the analyses should be laboratory replicates. Triplicates should be analyzed on one of every 20 samples or on one sample per batch if less than 20 samples are analyzed. The acceptable variation among replicates is 20 percent or less.

Tables B4-5 and B4-6 provide a brief summary of QA/QC for laboratory analyses. For information concerning detection limits, see Water Column Chemistry QA/QC Considerations (Section B2.4).

B4.5 Statistical Design Considerations

Statistical strategies may mitigate the high costs of collecting sufficient quantities of sediment. See statistical design considerations: composite sampling, power analyses, and power-cost analysis (Section B.3).

B4.6 Use of Data

The results may be used to monitor rates of recovery following environmental interventions, to evaluate the condition of benthic habitats, and to provide early warnings of potential impacts to the estuarine ecosystem. The singular use of sediment pollutant loading data to assess the condition of the benthic habitat or to guide the decision-making process is not recommended. Data acquired from monitoring sediment contaminant levels, in conjunction with the sediment's physical properties, may be used to assess the bioavailability of these pollutants (SCCWRP, 1986). Subsequent analysis of this information, in conjunction with biological data, may be used to assess

TABLE B4-5. SUMMARY OF QUALITY CONTROL SAMPLE

Analysis Type	Recommended Frequency of Analysis
Surrogate spikes	Required in every sample - minimum 3 neutral, 2 acid spikes, plus 1 spike for pesticide/PCB analyses, and 3 spikes for volatiles. Isotope dilution techniques (i.e., with all available labeled surrogates) is recommended for full scan analyses and to enable recovery corrections to be applied to data.
Method blank	One per extraction batch (semivolatile organics). One per extraction or one per 12-hour shift, which ever is most frequent (volatile organics).
Standard reference materials	<50 samples: one per set of samples submitted to lab. >50 samples: one per 50 samples analyzed.
Matrix spikes	Not required if complete isotope dilution technique used. <20 samples: one per set of samples submitted to lab. ≥20 samples: 5 percent of total number of samples.
Spiked method blanks	As many as required to establish confidence in method before analysis of samples (i.e., when using a method for the first time or after any method modification).
Analytical replicates	<20 samples: one per set of samples submitted to lab ≥20 samples: one triplicate and additional duplicates for a minimum of 5 percent total replication.
Field replicates	At the discretion of the project coordinator.

the impacts and risks of sediment pollutants to human populations, benthic populations/communities, and the estuarine ecosystem.

Monitoring of pollutant levels in sediments is a widely-accepted means of measuring the condition of the benthic habitat and is a powerful tool in the evaluation of spatial and temporal effects of anthropogenic and natural disturbance. However, for sediment chemistry data to be useful in interpreting toxicological response or in predicting environmental risks from sediment exposure, sediment criteria are needed. The

TABLE B4-6. SUMMARY OF WARNING AND CONTROL LIMITS FOR QUALITY CONTROL SAMPLE

Analysis Type	Recommended Warning Limit	Recommended Control Limit
Surrogate Spikes	10 percent recovery	50 percent recovery
Method Blank		
Phthalate, Acetone	30 percent of the analyte	5 µg total or 50 percent of the analyte
Other Organic Compounds	1 µg total or 5 percent of the analyte	2.5 µg total or 5 percent of the analyte
Standard Reference Materials	95 percent confidence interval	95 percent confidence interval for Certified Reference Material
Matrix spikes	50-65 percent recovery	50 percent recovery
Spiked Method Blanks	50-65 percent recovery	50 percent recovery
Analytical Replicates	–	±100 percent coefficient of variation
Field Replicates	–	–
Ongoing Calibration		25 percent of initial calibration

Washington State Department of Ecology (1991) has adopted sediment criteria and the EPA has recently published a sediment classification methods compendium (U.S. EPA, 1992). Both of these documents may provide useful guidance. Our ability to extrapolate from sediment chemistry data to predictions of ecological or human health risks is, however, greatly limited by our understanding of causal relationships between exposure and response.

B4.7 Summary and Recommendations

Rationale

- The sediments represent the ultimate repository for many anthropogenic contaminants infiltrating the estuarine environment

- The objective of monitoring bulk sediment chemistry is to detect and describe spatial and temporal changes of these sediment pollutants

Monitoring Design Considerations

- It is recommended that consistent types of sampling gear, and location and timing of sample collection be implemented to allow for comparisons among studies and over time

- Collection of undisturbed sediment requires that the sampler:
  - create a minimal bow wake when descending
  - form a leakproof seal when the sediment sample is taken
  - prevent winnowing and excessive sample disturbance when ascending
  - allow easy access to the sample surface in order that undisturbed subsamples may be taken

- Penetration well below the desired sampling depth is preferred to prevent sample disturbance as the device closes.

- In general, analysis of the upper 2 cm will allow examination of recent sediment contamination events, however, site-specific conditions and program objectives should be considered when a sampling depth is selected

- Total organic carbon normalization is recommended to allow comparisons of chemical residue concentrations between locations – normalizing the data will depend upon the objectives of the individual monitoring program

- It is recommended that sediments be sampled when contaminant concentrations are expected to be at their highest level

Existing Analytical Methods

- It is recommended that consistent types of analytical protocols be implemented to allow for comparisons among studies

- Laboratories should perform acceptably on Standard Reference Materials and intercalibration exercises before performing routine analyses

- Periodic full-scan analyses should be undertaken to identify any new pollutants which should be monitored

- Metals/Metalloids
  - The combination of GFAA and ICP are the recommended methods for the detection of metals and metalloids (U.S. EPA, 1987b)
  - cold vapor AAS is the recommended protocol for mercury detection

- Organics
  - GC/MS in conjunction with isotope dilution is recommended for the detection of semi-volatile organic compounds
  - vacuum super-cooled trap in conjunction with a purge and trap device is recommended for the detection of volatile organics (Hiatt, 1981)
  - isotope dilution option is recommended as it provides reliable recovery data for each analyte (U.S. EPA, 1986a)

QA/QC Considerations

- The primary criteria for acceptable field sampling equipment and protocols are the collection of undisturbed and uncontaminated environmental samples

- Adequate sampling containers and preservation techniques must be used and appropriate holding times followed to ensure the integrity of samples and their analyses

- Blank, spike recovery, and replicate analyses are recommended quality control checks

- A report describing the objectives of the analytical effort, methods of sample collection, handling and preservation, details of the analytical methods, problems encountered during the analytical process, any necessary modifications to the written procedures, and results of the QC analyses should be included with the analytical data

Statistical Design Considerations

- Compositing sediment sampling consists of mixing samples from two or more replicates collected at a particular location and time

- Space- (combining composites from several locations) and/or time- (combining several composites over time from one location) bulking strategies should be used judiciously since information concerning spatial and temporal heterogeneity may be lost

Use of Data

- The data garnered from monitoring of the pollutant levels in estuarine sediments should be used in conjunction with that collected from other physical and biological monitoring to monitor rates of recovery following environmental interventions, to evaluate the condition of benthic habitats, and to provide early warnings of potential impacts to the estuarine ecosystem

- The singular use of sediment pollutant loading data to assess the condition of the benthic habitat or to guide the decision-making process in not recommended

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B5.0 Plankton: Biomass, Productivity and Community Structure/Function

B5.1 Rationale

Although increased primary production resulting from intentional nutrient inputs has been shown to increase fish stocks in some experimental systems, the possible increase in fisheries in naturally productive systems is generally considered insignificant when compared to the deleterious effects of eutrophication. The most conspicuous and potentially detrimental effects of eutrophication to estuarine environments are increased turbidity, phytotoxins, and creation of hypoxic or anoxic conditions. Furthermore, changes in nutrient concentrations in an estuary may result in the potential for long-term biological changes in the estuarine community structure and function (e.g., changes in species distribution and abundance of both primary producers and consumers).

If eutrophic conditions are suspected of occurring, monitoring of the plankton community may assist in detecting and defining the spatial and temporal scales of these conditions. The objectives of monitoring plankton characteristics are to detect spatial and temporal changes in plankton community structure and function. These results may be used to assess the consequences of spatial and temporal variability in plankton community structure and function to higher trophic levels which depend upon planktonic resources. In addition, this information may be used to further the understanding of the relationship between water quality conditions and planktonic community structure and function.

B5.2 Monitoring Design Considerations

Sampling Methods

Phytoplankton – Plankton samples are frequently collected using water bottles. They are simple devices, usually consisting of a cylindrical tube with stoppers at each end and a closing device that is activated by a mechanical or electrical signal. Each samples a discrete parcel of water at any designated depth. Multiple samplers are fixed on a rosette frame in order that several depths may be sampled during one cast and/or that replicate samples may be collected at a particular depth. The most commonly used bottle samplers include the Kemmerer, Van Dorn, Niskin, and Nansen samplers (U.S. EPA, 1987a).

Phytoplankton samples should be collected at a variety of depths throughout the water column, some above and some below the pycnocline. To adequately characterize the phytoplankton community, it is recommended that a minimum of four depths in the vertical profile should be sampled: 1) one meter below the surface, 2) one meter above the bottom, 3) one meter above the pycnocline, and 4) one meter below the pycnocline. If the waters are too shallow or no stratification occurs, it would be appropriate to take the latter two samples at evenly spaced distances between the top

and bottom samples. For example, composite water column samples collected for the Virginia Chesapeake Bay Plankton Monitoring Program are taken at five different depths above and five different depths below the pycnocline (U.S. EPA, 1990). Vertical measures of chlorophyll a (an indirect measure of phytoplankton standing stocks) can be made by fluorometric or spectrophotometric determination of chlorophyll a from these depth samples.

If available, a pump station may be used with a flow-through fluorometer for a continuous profile of chlorophyll a concentration with depth (Lorenzen, 1966). Samples to be used for taxonomic analysis should be collected with water bottles, as agitation associated with pumping may damage cells, making them unidentifiable. Pumps should only be used for determination of chlorophyll a concentrations.

Zooplankton – Because zooplankton possess varying degrees of swimming ability, they have the potential for aggregating in patches or in a narrow depth strata. This introduces additional complications into quantitative sampling, such as avoidance of certain types of gear.

Until recently, the role of the microbial loop, which consists of bacteria, flagellates, ciliates and microzooplankton (<200 μm), has been overlooked. These organisms may represent a significant pathway in the reutilization and conversion of dissolved organic carbon into larger zooplankton and benthic organisms (Azam *et al.*, 1983; Pomeroy, 1984).

The sampling methods to be used for collecting zooplankton will vary depending on the size of the organisms. Microzooplankton (size range of 20-200 μm) can be collected with water bottles at various depths similar to those used for phytoplankton, or small (for example, 44 μm) mesh nets can be used (Jacobs and Grant, 1978). Pumping systems can also be used which have the advantage of being able to take samples integrated over depth and of collecting samples while the ship is underway (Beers *et al.*, 1967). However, pumps may damage soft-bodied organisms and they are more expensive and complicated than water bottles. Triplicate samples should be collected from each station depth, thus allowing for statistical analysis of intra-station variability.

For small mesozooplankton (greater than 200 μm) nets are generally used (UNESCO, 1968). Additional tows may have to be made with larger nets in order to collect representative samples of larger zooplankton and larval fish. All tows should be replicated. The number of replicates necessary for the desired precision of estimation should be determined during a preliminary or pilot sampling program. A number of other considerations, including net mouth diameter, towing speed and ship board handling of samples will affect sampling results. Some problems associated with the

use of nets for zooplankton sampling include avoidance and clogging which may result in underestimating abundance and diversity and loss of filtration efficiency (McGowan and Fraundorf, 1966; Wiebe and Holland, 1968).

Conductivity-Temperature-Depth (CTD) Systems

Continuous vertical profiles of chlorophyll a concentrations can be obtained by attaching a fluorometer to a conductivity, temperature and depth (CTD) system. An additional sensor measuring dissolved oxygen could be attached to the CTD system. This combined instrument package will make direct measurements of chlorophyll a levels and the degree of eutrophication and water column stratification.

The advantage of this measurement system is that instead of pulling water up from depth to a measuring device on the deck of a ship, the instrument is lowered through the water column taking samples in undisturbed water. Sampling rates can be as high as 24 samples per second with a descent speed of 1 meter per second. Data is usually collected by a computer-based data acquisition system and in advanced systems can be displayed in real-time as it is being collected. A rosette water sampler can be lowered at the same time as the CTD package to collect water samples at discrete depths. These samples will be used for taxonomy analysis and to verify and calibrate chlorophyll a measurements from the CTD fluorometer. The calibration will be carried out using laboratory spectrophotometer methods.

Selection of Spatial and Temporal Sampling Strategies

The time of the year should be controlled or stratified in the design – the use of annual averages is seldom good practice. Given the seasonal variation characteristic of phytoplankton abundances, it is recommended that direct comparisons between samples collected during different seasons be avoided. Studies investigating interannual variation in the characteristics of phyto- and zooplankton communities should be conducted during the same season (preferably the same month) each year.

Due to their short turnover times, phytoplankton communities are capable of responding to perturbations much more rapidly than other biotic groups. Therefore, phytoplankton samples should be collected relatively more frequently. In those situations where phytoplankton communities display pronounced seasonal variations in standing stock or production, it may be appropriate to use a temporally stratified sampling approach. For example, in the Maryland Chesapeake Bay Phytoplankton Monitoring Program, sampling takes place once monthly from October through March and twice a month from April through September (U.S. EPA, 1990).

The lifespan of zooplankton on the other hand is longer than phytoplankton, so the capacity for responding to perturbations is less than that of phytoplankton. Therefore less frequent sampling is required. As with the phytoplankton community, the zooplankton monitoring program should consider the natural temporal fluctuations in abundance and species composition.

The distribution of planktonic organisms is strongly influenced by currents and tides. Sampling programs should be developed to reflect the patchiness caused by tidal fronts, and regions of convergence or divergence of currents within the estuary.

In many cases, regular monitoring of the zooplankton community may not be necessary unless changes in the phytoplankton community are observed which would induce changes in the herbivore community. Because many zooplankton graze on phytoplankton, in areas where the phytoplankton community has been affected, alterations of the zooplankton community are a distinct possibility. In other cases, monitoring of zooplankton may be desirable only when there is evidence of previous impact on the zooplankton community, or in those situations where point and nonpoint source discharges are located in areas where there is high potential impact on zooplankton. For example, it may be useful to monitor zooplankton in estuarine environments with macroplanktonic larvae of important commercial or recreational species.

Taxonomic Identification and Enumeration

Subsamples drawn from water collected in water sampling bottles should be preserved for later microscopic analysis to determine phytoplankton community composition. The choice of fixation will depend on the dominant types of phytoplankton known to inhabit a given area (buffered formaldehyde and Lugol's solution are two common fixatives). Preserved phytoplankton samples normally must be concentrated for quantitative microscopic analysis.

The taxonomic analysis should include identification and enumeration of dominant phyto- and zooplankton taxa and measures of population abundance whenever possible. Particular attention should be given to meroplanktonic larvae of commercially, recreationally or ecologically important species.

The large number of species, incomplete taxonomy, and small size of the organisms comprising the microbial loop make taxonomic identification extremely difficult. Ecologists working with these groups tend to concentrate on higher taxonomic levels and functional groupings. Staining with Acridine Orange, or similar stain, and direct counting with epifluorescence microscopy has been used to estimate bacterial numbers

(Harvey and Young, 1980; Wright and Coffin, 1983) and microprotozoan abundances (Sherr and Sherr, 1983).

B5.3 Existing Analytical Methods

Perturbations to the phyto- and zooplankton communities should be analyzed in relation to other potential impacts on other biological communities which include:

- potential primary and secondary impacts to higher trophic level communities (e.g., food web impacts)
- occurrence of toxic or nuisance phytoplankton

Some CCMPs may require that both larval and adult zooplankton community structure and function should be analyzed in order to adequately assess impacts to high trophic levels. Standardized methods are recommended to permit comparisons between studies.

Biomass and Productivity

Methods for estimating phytoplankton biomass used in earlier monitoring surveys include cell counts, total cell volume estimates, protein estimates, and dry weight. These methods have certain disadvantages related to speed of the technique (e.g., time-consuming) and the degree of accuracy for the estimation of phytoplankton biomass (D'Elia *et al.*, 1986). The use of chlorophyll a measurements, especially fluorometric, has become widespread primarily because the method is relatively fast, simple and reproducible.

Phytoplankton biomass can be indirectly measured through the measurement of the concentration of chlorophyll a in the water. This is done through fluorometric or spectrophotometric measurements. Within these methods there are also differences in extraction techniques for chlorophyll determination, including various methods of filtration, solvents, temperature and/or physical treatment (sonication or grinding) (D'Elia *et al.*, 1986).

However, with both the fluorometric and spectrophotometric determinations, the presence of accessory pigments may interfere with determination of chlorophyll a. Several researchers, however, have successfully used chromatographic procedures to separate interfering substances prior to determination (D'Elia *et al.*, 1986). The High Performance Liquid Chromatography (HPLC) is generally acknowledged as the most

accurate (and most expensive) method for chlorophyll determinations (Bidigare, 1989).

UNESCO (1973) recommends that the ^{14}C light-dark bottle technique be used to estimate primary production. This technique is more sensitive than, and requires shorter incubation times than the O_2 light-dark bottle method. However, the O_2 method measures gross primary production, net primary production and respiration, using inexpensive laboratory reagents, while the ^{14}C technique estimates only net primary production and requires specialized training and equipment, and relatively expensive radioisotopes.

Biological Indices

Biological indices allow large amounts of multivariate data to be reduced to a single number. The numbers of individuals and the numbers of species have been found to be good indicators of anthropogenic disturbance, as well as of other environmental stresses. Furthermore, these simple biological indices are less ambiguous and are often as informative as diversity indices (U.S. EPA, 1985; Green, 1979; Hurlbert, 1971).

More complicated indices — e.g., species diversity, species richness, dominance, evenness — have found varying degrees of acceptance. Diversity indices have the following limitations (Green, 1984):

- often lack biological meaning
- are not robust empirical indicators of any important correlates of "estuarine health"
- do not incorporate information of form and function of resident species
- are susceptible to biases associated with well-described taxa

However, species diversity indices are a widely used measure of community structure.

The dominance index is a measure of the degree to which one or a few species dominate the community. The dominance index, herein defined as the minimum number of species required to account for 75 percent of the total number of individuals, has been useful in describing community structure (Swartz *et al.*, 1985). It is easily calculated, does not assume an underlying distribution of individuals among species, and is statistically testable.

Indicator Species

Abundances of selected indicator species can be used to evaluate response of the community as a whole. Examination of abundances of individual indicator species are generally informative and may reduce the cost of the analysis. The absence of pollution sensitive species and the enhancement of opportunistic and pollution tolerant species may assist in defining the spatial and temporal extent and magnitude of impacts. However, indicator variables must possess the following characteristics (Green, 1984):

- provide sufficiently precise and accurate appraisals of:
  - species of concern
  - anthropogenic disturbances to planktonic communities
  - presence/absence or the magnitude of anthropogenic perturbance to the estuarine ecosystem

- cost-effective and a statistically reliable alternative to monitoring all critical planktonic community measures of habitat perturbance

- appropriate for the spatial and temporal scale demanded by the study objectives

It is recommended that multiple pollution-tolerant and pollution-sensitive indicator species are selected by clearly defined criteria. Multiple indicator species often provide a more complete representation of environmental conditions.

B5.4 QA/QC Considerations

Variability in measurements caused by field heterogeneity is quantitatively determined by the analysis of replicate field samples. Replicate sampling should be conducted at all field stations where measurements are to be used in comparisons. Analysis of replicate sample data is necessary for assessing the reliability of such comparisons.

Laboratory performance and calibration should be verified at the beginning and periodically during the time analyses are performed. Commercially available chlorophyll is recommended for use in calibration. Chlorophyll quality control samples are available from EPA's Environmental Monitoring and Support Laboratory in Cincinnati, Ohio. Use of blind, split or other control samples can be used to evaluate performance. The "Interim Guidance on Quality Assurance/Quality Control (QA/QC) for the Estuarine Field and Laboratory Methods" (U.S. EPA, 1985) provides a standard operating procedure for chlorophyll measurements.

B5.5 Statistical Design Considerations

Consideration of statistical strategies will mitigate the high costs of collecting and processing samples. Also see Statistical Design Considerations: Statistical Power Analysis and Power-Cost Analysis (Appendix B Introduction; Section B.3).

Temporal Stratification of the Data

Temporal stratification of the data should not be attempted until sufficient knowledge of long-term natural cycles is attained. Initially, simple regression analyses may be conducted on seasonally stratified data in order to identify monotonic temporal trends. Further examinations of whether conditions are improving or degrading over time may be conducted using statistical time series analyses (e.g., temporal autocorrelation, spectral analyses, etc.)

Information derived from water quality monitoring will be important in interpreting the results of plankton sampling. For example, phosphate concentrations may indicate the cause of a phytoplankton "bloom," while measures of dissolved oxygen levels may describe the consequences of the "bloom" to other living estuarine resources. Therefore, the selection of water quality and plankton sampling strategies must not be done independently. These programs should be integrated to the fullest extent possible to allow correlation of observed responses to changes in water quality parameters. Also, alterations to the plankton community should be analyzed in relation to other impacts on biological resources such as food web impacts on fish communities.

B5.6 Use of Data

Plankton monitoring strategies should be able to delineate between natural variability in plankton stocks and those caused by anthropogenic changes in nutrient concentrations.

Characterization of phytoplankton species abundance, distribution and primary productivity provide indications of water quality conditions. Monitoring changes in phytoplankton community composition and densities are critical for the interpretation and evaluation of long-term trends in water and habitat quality. Further understanding of the causes of excessive water column and sediment oxygen demand requires tracking of photosynthetic activity and metabolic rates over time (Chesapeake Executive Council, 1988). Zooplankton abundance and distribution are affected both by changes in phytoplankton and changes in predator populations. Therefore, population characteristics of this group can indicate symptoms of water quality problems, fishing pressure and other habitat problems for predator species (Chesapeake Executive Council, 1988).

B5.7 Summary and Recommendations

Rationale

- Track phytoplankton and herbivore populations if eutrophication is suspected
- Purpose of monitoring is to assess the effectiveness of the CCMP in mitigating the potential impacts caused by changes in the planktonic community biomass and structure/function

Monitoring Design Considerations

- Collect both phytoplankton and zooplankton samples at specific depths throughout the water column
- Phytoplankton samples taken with water bottles and/or pumps can be used for estimation of chlorophyll concentrations
- Zooplankton sampling methods based on size of target organisms
- Monitoring programs should consider natural and temporal fluctuations in plankton biomass and species composition
- Taxonomic analysis should include identification and counts of dominant species
- Other components of the overall monitoring program, including water quality and fish and shellfish communities, should be integrated with plankton community data to establish relationships and trends
- Selected biological indices should retain biological meaning, be robust indicators of estuarine "health", and incorporate species form and function
- Indicator species should possess the following characteristics:
  - sensitive to planktonic perturbances of concern
  - cost-effective and statistically reliable alternative to measuring all species in a monitoring program
  - statistically reliable indicative measures of habitat perturbance
  - appropriate for the spatial and temporal scale determined by the study objectives

- Selection of reference sites is key to the evaluation of environmental impact due to anthropogenic perturbances; several reference sites may be required to provide proper control for sampling sites

Existing Analytical Methods

- It is recommended that consistent types of sampling gear, and location and timing of sample collection be implemented to allow for comparisons among studies

- Chlorophyll can be estimated using fluorometric or spectrophotometric methods using a variety of filtration and extraction techniques

- Phytoplankton productivity can be measured using the ^{14}C light-dark bottle technique or O_2 light-dark bottle method

QA/QC Considerations

- Replicate samples should be taken at all field stations where applicable

- Laboratory performance evaluations and calibrations must be done on a regularly scheduled basis

- Standard chlorophyll samples, obtained from the EPA, should be used for calibrations

Statistical Design Considerations

- Power analyses may be applied to determine the appropriate number of sample replicates required to detect a specified difference

- Temporal integration of the data should not be attempted until sufficient knowledge of long-term natural cycles is attained

Use of Data

- Detect short-and long-term spatial and temporal trends in overall biomass and productivity

- Detect short- and long-term spatial and temporal trends in species abundance, distribution and composition
- Examine relationship between water quality conditions and trends in plankton community characteristics
- Examine relationship between plankton community characteristics and impacts on other living resources (e.g., fish and shellfish communities)

B5.8 Literature Cited and References

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B6.0 Aquatic Vegetation

Human activities that contribute the greatest direct impacts to vegetation loss and modification are those that cause physical alterations to the habitat. Physical alterations may cause habitat loss directly or indirectly by modifying natural processes that significantly affect aquatic vegetation, such as freshwater inflow, hydrology, sedimentation, and sea-level changes.

Marine and estuarine vegetation may be organized into the following groups:

- Emergent vegetated wetlands
  - marshes
  - mangroves
- Submerged aquatic vegetation (SAV)
  - seagrass beds
  - other submerged plant communities
- Macroalgal communities

Although all of these habitat types provide essential functions for living marine resources, the focus of this discussion is primarily on emergent vegetated wetlands due to the recognized ecological value of these habitats and their rapid rate of deterioration and loss in many coastal areas.

TABLE B6-1. LIST OF TERMS

Emergent Vegetation	=	erect, rooted, herbaceous vegetation
Function	=	the physical, chemical, and biological processes or attributes of a habitat without regard to their importance to society
Submerged Aquatic Vegetation	=	aquatic plants that grow while wholly submerged and anchored to the substrate
Values	=	processes or attributes that are valuable or beneficial to society
Wetland	=	those areas that are inundated or saturated by surface or ground water at a frequency and duration sufficient to support, and that under normal circumstances do support, a prevalence of vegetation typically adapted for life in saturated soil conditions. Wetlands generally include swamps, marshes, bogs, and similar areas.

B6.1 Rationale

Declines in the quality and quantity of marine habitats are thought to reduce production of living marine/estuarine resources and diminish other important values of habitats. Marine and estuarine habitats perform important functions for all living marine resources. Aquatic vegetation is used by many species for spawning, rearing, feeding, migration, and shelter from predators. A positive correlation generally exists between wetland and submerged aquatic vegetation (SAV) acreage and the abundance of commercially and recreationally important living marine resources (e.g., fish and crabs). Populations of living marine resources therefore appear to be limited by the quantity and quality of aquatic vegetation. In fact, the condition of wetlands may prove to be an effective ecological indicator of the condition of selected bird and mammal populations.

Furthermore, marine and estuarine habitats, in particular vegetated wetlands (tidal marshes) and submerged aquatic vegetation, perform other valuable functions to man and the environment. These functions include erosion and flood protection, water quality control, and provision of habitats for waterfowl and wildlife. Pressures brought on by population shifts to coastal areas and associated industrial and municipal expansion will continue to jeopardize coastal wetland resources. Monitoring changes in aquatic vegetation will permit assessment of impacts to the habitat and the organisms it supports.

B6.2 Monitoring Design Considerations

It is essential to understand the effect of monitoring design characteristics on the results obtained and to standardize them as much as possible to ensure the comparability of monitoring efforts throughout the estuary.

Levels of Monitoring Effort

One, or some combination of the following four levels of monitoring effort may be chosen:

The lowest level of effort would involve periodic measurement of the areal extent of wetlands based on small scale aerial photography. Aerial photography may also be used to estimate the extent of submerged aquatic vegetation in low turbidity areas; in areas of high turbidity, extensive ground truthing would probably be necessary.

The next level of detail would be to map the extent of the dominant plant species. Many of the dominant species are readily identifiable from aerial photographs. For

example, *Spartina alterniflora* has stiff upright stalks, while *Spartina patens* is more flexible and tends to bend over during low tide.

A third level might include on site measurements relating to functional aspects of the system. For example, estimates of underground and aerial biomass of dominant plants may provide information on primary production and health of the system. Kibby *et al.* (1980) discuss various techniques for evaluating emergent vegetation. These techniques may be readily modified to assess submerged vegetation.

Level four monitoring would involve integrated studies that examine many functional aspects of the wetland or submerged aquatic vegetation and the associated animal community. Techniques such as the Minimal Habitat Matrix, Wetland Evaluation Technique and Habitat Evaluation Procedure, discussed in Section B6.3, would be appropriate.

Sampling Period

The aerial coverage and bed density for SAVs vary from year to year due to catastrophic storms, exceptionally high precipitation and turbidity, and other poorly understood natural phenomena. Therefore, short term monitoring will not provide an accurate assessments of the trend in SAV coverage, but may rather reflect the impacts of infrequent or periodic events. In addition, wetland losses in acreage occur at low enough frequencies that yearly measurements are impractical. Furthermore, the types of wetland losses that are now occurring appear to be moving away from larger increments to a smaller form of wetland loss that may fall within the margin of error for current detection limits.

Seasonal/Daily Considerations – In the conduct of habitat functional assessments, seasonal variations have to be considered. SAV and wetland sampling should be conducted during the period of peak biomass. Other factors (e.g. tides, weather conditions, turbidity) combined with a limited growing season also make for a finite window of opportunity for data capture.

B6.3 Existing Analytical Methods

Spatial Trends

The most straightforward approach for assessing trends in the aquatic habitat – and the one most typically used – is to document the loss of habitat acreage (Table B6-2; France and Hedges,1989). Spatial data alone are particularly important because of the

public concern over declining habitat acreage (both wetlands and submerged aquatic vegetation) in the nation's estuarine areas. Good recent information on habitat locations and boundaries form a baseline for monitoring future changes. However, delineating the extent of habitats alone does not provide a complete measure of the quality of the habitat in terms of its value for fish, bird and marine mammal populations.

TABLE B6-2. LIST OF ANALYTICAL METHODS

Spatial Trends
- Acreage Loss

Functional Trends
- Minimum Habitat Matrix
- Wetland Evaluation Technique
- Habitat Evaluation Procedure

Losses of aquatic vegetation acreage in an area of particular concern may be the compelling reason for the evaluation of this habitat within the estuary. The primary sources of spatial information are maps, aerial photography, and remote or satellite imagery. These tools can serve as a simple visual aid for communicating the problem to the public as well as a main data source for wetland structural and functional characteristics and assessment of cumulative impacts and trends. Aerial or remote monitoring can measure changes in acreage, cover types, drainage, configuration and other spatial parameters related to overall habitat condition and health.

Maps – Maps are the least expensive spatial data source and are also the most simplified. Generally, maps do not capture all visible characteristics that can be observed from aerial photographs, and rather selectively portray a subset of what is in an area. However, maps such as U.S. Geological Survey Topographic Quadrangle maps or U.S. Fish and Wildlife Service National Wetlands Inventory (NWI) maps can be used as a base of information from which to compare changes in wetland acreage.

The main benefits of maps for identifying habitats is in their ease of use and low cost. Their main disadvantage is that habitats are often not clearly or accurately delineated on maps, and if they are, they are generally not updated frequently enough to provide timely information on trends. For instance, although the Fish and Wildlife Service has mapped over 90% of the wetlands in the coastal zone of the lower 48 states, many of these maps are over 10 years old.

There have been efforts by some states and the Federal Government to develop high-resolution automated databases of habitat types through a process of digitizing NWI maps at a resolution of approximately one acre. The utility of high-resolution and geographically referenced databases for resource managers has been evaluated by U.S. Fish and Wildlife Service (Dahl, 1987). Digitizing NWI maps would make updating information on the status and trends of habitat types easier and more accurate; however, the progress toward digitizing all coastal wetland maps has been hampered by the time-consuming and costly nature of the process involved.

Aerial Photography – The most broadly used source of habitat spatial information is aerial photography. Aerial photographs can be produced at a range of scales and spatial resolution. Small scales can be used when, for example, very fine detail is desired for mapping vegetation cover types or complicated drainage patterns. Large scale coverage can be used when a broad area of coverage is desired. In addition, color infrared photography can be used to detect differences in soil moisture and subtle differences in vegetation types accentuating the upland/wetland boundary.

Existing aerial photographs are inexpensive. However, new overflights can be expensive and are often complicated, requiring professional assistance. The advantage of aerial photographs is that they generally contain more detail than maps of the same scale or than satellite imagery. When planning overflights for aerial photography, the following factors must be considered:

- tides
- weather conditions
- time of day (affects sun angle, atmospheric haze and cloud cover)
- turbidity

Photographs from new overflights can be compared to base maps or older aerial photographs to record changes over time.

Remote Sensing/Satellite Imagery – Satellite imagery is a useful source of spatial data when very large study areas are involved and fine detail is not important. Although expensive, when large study areas are involved, satellite imagery may become more economical than aerial photography. Other than cost, a significant constraint in the use of satellite imagery is the lack of resolution that could result in missing small

habitats or features; remote sensing provides a coarse estimate of size or boundaries. However, if the area under investigation is characterized by a very large homogenous habitat, this constraint may not be as important.

Because images are acquired by scanners and stored in digital form, the spectral properties, brightness values and other digitally stored data can be manipulated to enhance or reveal characteristics (such as productivity) not always visible from aerial photography. However, factors affecting water clarity, such as suspended sediments, may affect these spectral properties. These factors, and the amount of cloud cover over the estuary, should be considered when appraising the use of remote sensing. Ground-truthing of satellite images is highly recommended in order that accurate interpretations of the images may be achieved.

When analyzing remotely sensed images, the availability and effectiveness of appropriate algorithms may be limited (Platt and Sathyendranath, 1988). Interpretation, manipulation, and analyses of remotely sensed images requires persons with specialized skills handling image analyzing software and hardware. Furthermore, some habitat assessment methods require a high degree of advanced understanding of the science involved in the assessment, such as plant taxonomy or hydrology. The selection of the assessment method will therefore depend on the level of specificity required to conduct the assessment and the available technical expertise.

Transects and Quadrats – Physical and biological attributes of wetland vegetation may be taken at regular intervals along a transect: point intercept method (SCS, 1976). Critical biological characteristics include species name, plant and mulch biomass, and foliar and basal cover. Physical characteristics (e.g., salinity, water depth, flow rates) should also be collected to assist in the interpretation of the biological data.

Plants may be harvested from standard-sized quadrats (e.g., 1 m^2) along a transect (SCS, 1976). Conversion factors are available to convert plant species data from biomass per plot to more common measures of productivity (e.g., pounds/acre or kilograms/hectare). Measures should specify green weight or air-dry weight; factors for converting between green weight to air-dry weight exist.

Measures of cover are fundamental to managing living resources of wetlands. Basal cover at the erosion interface provides information concerning retention of detrital material and potential for erosion. Basal cover complements measures of productivity by providing information concerning the stability of the habitat. For example, although a plant community may be highly productive, it may be susceptible to erosion

if there exists little basal cover at the erosion interface. This information may be used to guide implementation of erosion control measures.

The U.S. Department of Agriculture (USDA) has developed the Range Data System (RDS), a nationwide database system for grass-based ecosystems. RDS is capable of storing, retrieving, and analyzing data on wetland plant productivity and species composition. RDS also provides a remarks field where data such as salinity and water depth may be recorded.

Functional Trends

The purpose of assessing habitat functional trends is to compare the relative value of one habitat to another or to assess changes in values of the same habitat over time. Detailed, site-specific, functional assessment studies can be expensive, time-consuming, and often impractical when time or budgetary constraints exist. Although more expensive, correlating functional habitat losses to acreage loss is a more meaningful measure of the condition of estuarine resources than acreage loss alone.

Where time and budget allow, implementation of detailed, carefully designed monitoring programs or use of quantitative computer models usually give superior-quality results (Table B6-2). All functional assessment methods, however, are limited by the level of understanding of the actual processes taking place in habitats, which vary with habitat type, region of the country, and physical, chemical and biological conditions. Functional assessments usually describe trends in water quality, hydrology, and biota that are potentially attributable to habitat loss and impacts.

Standardized, relatively rapid functional evaluation procedures generally provide stronger replicability and technical comprehension. Two of the most frequently standardized evaluation techniques are the Wetland Evaluation Technique (WET), originally developed for the Federal Highway Administration and later revised for the U.S. Army Corps of Engineers (Adamus *et al.*, 1987) and the Habitat Evaluation Procedure (HEP), developed by the U.S. Fish and Wildlife Service (U.S. FWS, 1980). Many of the more recently developed habitat evaluation methods are modified versions of these techniques.

Minimum Habitat Matrix – As part of the Chesapeake Bay Program, minimum habitat guidelines for various species are developed with the ultimate goal of reestablishing a balanced ecosystem. This method is designed to provide information on the minimum habitat quality needed by a target species and identifies those factors (both

environmental and ecological) required for the species. This information is formatted into a habitat requirement matrix that defines the habitat parameters needed for successful reproduction and survival of the indicated species.

The matrices can indicate the vital environmental parameters that should be monitored and thus facilitate the establishment of monitoring programs. This process is used to estimate the feasibility, benefits and potential costs of maintaining and protecting an estuarine environment suitable for the successful reproduction and survival of aquatic vegetation (Chesapeake Executive Council, 1988).

One potential problem with this method is that only the target species and those organisms that the target species depends on for food are tracked, with the intention of maintaining habitat quality for both groups. This method may not completely recognize the complex species interdependence within estuarine environments.

Wetland Evaluation Technique (WET) – The Wetland Evaluation Technique outlines the procedure for conducting an assessment of the following wetland functions and values:

Ground Water Recharge	Production Export
Ground Water Discharge	Wildlife Diversity/Abundance
Flood Flow Alteration	Aquatic Diversity/Abundance
Sediment Stabilization	Recreation
Sediment/Toxicant Retention	Uniqueness/Heritage
Nutrient Removal/Transformation	

WET assesses the suitability of wetland habitat for 14 waterfowl species groups, 4 freshwater fish species groups, 120 species of wetland-dependent birds, 133 species of saltwater fish and invertebrates, and 90 species of freshwater fish. WET does not evaluate any other important wildlife resources (e.g., game and furbearing mammals). Other evaluation methods must be used to evaluate these other wildlife resources (Adamus *et al.*, 1987).

WET assesses functions and values by characterizing a wetland in terms of its physical, chemical, and biological processes and attributes (Adamus *et al.*, 1987). This characterization is accomplished by identifying threshold values for predictors. Predictors are simple, or integrated, variables that directly, or indirectly, measure the physical, chemical, and biological processes or attributes of a wetland and its surroundings.

WET evaluates functions and values in terms of social significance, effectiveness, and opportunity (Adamus *et al.*, 1987). Social significance assesses the value of a wetland to society due to its special designations, potential economic value, and strategic location. Effectiveness assesses the capability of a wetland to perform a function due to its physical, chemical or biological characteristics. Opportunity assesses the opportunity of a wetland to perform a function to its level of capability.

Habitat Evaluation Procedure (HEP) – The Habitat Evaluation Procedure is a method that can be used to document the quality and quantity of available habitat for selected wildlife species. HEP provides information for two general types of wildlife habitat comparisons: (1) the relative value of different areas at the same point in time; and (2) the relative value of the same area at various points in time. By combining the two types of comparisons, the impacts on, or improvement in habitat quality as a result of proposed or anticipated land and water changes on wildlife habitat can be quantified (Lonard and Clairain, 1986).

In the evaluation procedure, the habitat quality for selected species is documented based on an evaluation of the ability of key habitat components to supply the life requisites of the selected species. The evaluation involves using the same key habitat components to compare existing habitat conditions and the optimum conditions for the species of interest (U.S. FWS, 1980).

There are a number of limitations to using a habitat approach, such as HEP, in an evaluation system as was pointed out by the U.S. Fish and Wildlife Service (1980). Using habitat quality as an evaluation standard limits the application of the methodology to those situations in which measurable and predictable habitat changes are an important variable. It also forces a long-term "averaging" type of analysis. There is no assurance that populations will exist at the potential levels predicted by habitat analysis, as the analysis may not include all of the environmental or behavioral variables that may limit populations below the predicted habitat potential. In addition, socio-economic or political constraints may prevent the actual growth of certain populations to these levels (U.S. FWS, 1980).

B6.4 QA/QC Considerations

Whichever monitoring method is selected, the researcher should ensure that it has a high degree of replicability. All functional assessment methods contain assumptions that impact the results of the assessment. Some assessment methods also require excessive judgement on the part of the researcher and as a result, different users may arrive at different results. In selecting a method, the researcher should first determine whether assumptions are based on scientific knowledge or on the opinion of the author.

Before applying a method extensively within a region, the researcher should field test the method by comparing its ratings to those determined independently by long-term research studies of representative habitat sites. Ground-truthing remotely sensed and aerial images is highly recommended in order to ensure accurate interpretation of the images.

B6.5 Statistical Design Considerations

In designing an aquatic vegetation monitoring program the researcher must first determine the resources available. This will determine the extent of the monitoring program and the level of detail it can provide. If resources are limited, the extent of the monitoring program may only involve an assessment of the aereal extent of wetlands. If more resources are available and it is decided to undertake a functional assessment, the researcher must decide which functions are of greatest interest in the study area or which will provide the most information concerning the habitat attributes perceived to be of greatest value to the area.

Due to time and budgetary constraints, it may not be possible to evaluate all of the habitats within the region of concern (e.g., an estuary). In such cases, the researcher may choose to evaluate only a select few habitats. These may be those with potentially the greatest ecological value, those undergoing remediation, or those which are undergoing significant loss or modification.

Most habitat evaluation techniques involve some form of ranking, either quantitative or qualitative. The purpose of the monitoring in a estuary program is to periodically compare baseline conditions to conditions after the management plan has been implemented. In terms of habitat monitoring this means comparing the baseline acreage or functional rating of the habitat to conditions some time after the management plan has been implemented. Therefore, replicability of results is imperative.

See also Statistical Design Consideration: Statistical Power and Power-Cost Analysis (Section B.3).

B6.6 Use of Data

The results of aquatic vegetation evaluation will track trends in habitat quality and quantity so that they may be projected into the future. Additionally, changes in land use or other anthropogenic activities over time can be measured in terms of their impact upon aquatic habitats. It is a primary concern to accumulate an adequate set of data to establish a baseline so that any changes in habitat quantity and quality that are derived from mitigation, regulation or an estuary management program can be assessed. Historical data can chart changes in land use leading up to the present state.

The tracking of habitats may be an effective method of monitoring estuarine bird and mammal populations. These organisms are highly mobile and their presence or absence at particular monitoring stations may not be significant – individuals may be situated in another similar location. However, monitoring habitats which these organisms require (e.g., to nest, breed, feed, use for shelter from predators, etc.) may be an effective means of monitoring the threat to their well-being in the estuary. Loss of biologically critical habitats will indicate the potential decline or loss of estuarine bird and mammal populations.

Although single coastal development projects that result in modification or loss of estuarine vegetation may not have serious immediate environmental repercussions beyond the point of initial impact, many such projects in succession over time and over a broad geographic area may have far-reaching ecological consequences (Day *et al.*, 1989). Without reasonably accurate information on long-term and cumulative impacts of human activities on marine and estuarine habitats, good decisions on permitting, mitigation, or management of fisheries and wildlife will be difficult.

B6.7 Summary and Recommendations

Rationale

Monitoring habitat quantity and quality will provide information to assess impacts on living resources and to gauge the effectiveness of mitigation, regulatory and management programs.

Monitoring Design Considerations

- Possible resilience of habitat functions should be considered; need to be able to assess long-term and cumulative impacts
- Seasonal and daily variations may have an impact on habitat functions and the ability to identify habitat boundaries and thus affect evaluation results
- Some methods require a high degree of technical understanding while other methods can be easily understood and implemented with more limited technical understanding

Existing Analytical Methods

- Analyses of spatial trends is the most straightforward approach for assessing trends in the aquatic habitat. Typically, documentation of habitat acreage loss is assessed.

- Maps have the following properties
  - least expensive
  - easy to use
  - most simplified: do not capture all features
  - habitats not always clearly or accurately delineated
  - often not updated on regular basis

- Aerial photography has the following properties
  - range of scales and spatial resolution
  - color infrared photography to detect differences in soil moisture and vegetation types
  - relatively inexpensive
  - contain more detail than maps or satellite imagery

- Satellite imagery has the following properties
  - useful when large study areas involved and great detail not important
  - spectral properties, brightness values and other digitally stored data can be manipulated to enhance characteristics
  - expensive
  - lack of resolution found in aerial photographs

- Functional analysis considerations
  - provide more detailed information concerning the quality of habitat
  - can be expensive and time consuming
  - limited by the level of understanding of actual processes
  - WET and HEP two of the most frequently used standardized evaluation techniques

QA/QC Considerations

- Need high degree of replicability within a method

- Method should be field tested before being applied extensively

Statistical Design Considerations

- Available resources
- Determine which functional assessments will provide information concerning attributes of greatest value to the area
- Determine areal extent of monitoring program
  - entire watershed
  - select habitats with greatest potential value or undergoing significant loss or modification

Use of Data

- Project future trends in habitat quality and quantity
- Evaluate severity of habitat impacts from changes in land use or other anthropogenic activity over time
- Assess improvements in habitat quality and quantity resulting from mitigation, regulation, and estuary management programs
- Ecological indicator of potential decline or loss of estuarine bird and mammal populations

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B7.0 Benthic Infauna Community Structure

B7.1 Rationale

The objective of monitoring the benthic infauna is to detect and describe spatial and temporal changes in the structure and function of benthic communities. These results can be used to assess the condition – the "health" – of benthic habitats, to monitor rates of recovery following environmental interventions, and, since the benthic infauna represent a significant food source for many estuarine organisms, to provide an early warning of potential impacts to the estuarine ecosystem.

The benthic habitat may broadly be divided into hard- and soft-bottom habitats. Since the predominant habitat in estuaries is soft-bottom, assessment of soft-bottom community structure will be the emphasis of this section.

Monitoring the abundance of individual organisms in the benthic infauna is a widely-accepted means of measuring the condition of the benthic habitat (Bilyard, 1987). Benthic infaunal organisms are exceptional indicators of benthic conditions since:

- they are generally sedentary – observed effects are in response to local environmental conditions
- they are sensitive to habitat disturbance – communities undergo dramatic changes in species composition and abundance in response to environmental perturbations
- they often mediate the transfer of nutrients and toxic substances in the ecosystem – via bioturbation and as important prey organisms

Furthermore, monitoring benthic infauna is one of a handful of methods which provides *in situ* measures of biotic health and habitat quality. The assessment of benthic infaunal community structure is a powerful tool in the evaluation of spatial and temporal effects of anthropogenic and natural disturbances.

B7.2 Monitoring Design Considerations

The level of effort required to assess benthic community structure is relatively high. A field survey is required to collect organisms; sorting, identifying, and enumerating specimens requires labor-intensive and generally expensive processes. Evaluations of benthic community structure and function may also be costly and time-consuming.

The results of benthic monitoring programs can vary substantially depending on the objectives and corresponding design specifications. The characteristics that are primarily responsible for the variability in the results are:

- type of sampling gear
- sample sorting protocols
- level of taxonomy
- location and timing of sample collection

It is essential to understand the effect of these design characteristics on the results and to standardize them as much as possible to ensure the comparability of monitoring efforts throughout the estuary.

Analyses of power-cost efficiencies are essential in selecting the appropriate sampling gear and sample processing protocols. Ferraro *et al.* (1989) provide an example of power-cost analyses.

Sampling Devices

Sample collection protocols influence all subsequent laboratory and data analysis; it is key that benthic samples be collected using acceptable and standardized techniques. Several types of devices can be used to collect benthic macroinvertebrate samples: trawls, dredges, grabs, and box corers (McIntyre *et al.*, 1984). Many of these devices sample the benthos in a unique manner. Accordingly, conducting comparisons among data collected using different devices is inadvisable. It is recommended that the same area and volume of the sediment be sampled since different species of benthic macroinvertebrates exhibit different horizontal and vertical distributions (Elliot, 1971).

Furthermore, collection of sediments and benthic organisms should be done concurrently in order to mitigate the costs of field sampling and to permit sound correlation and multivariate analyses (see Sections B.3 and B.4). Therefore, it is recommended that the sampling device also be suitable for sampling the sediment (Table B7-1).

Collection of an acceptable sediment sample for infaunal analyses requires that the sampler:

- create a minimal pressure wave when descending
- form a leakproof seal when the sediment sample is taken
- prevent winnowing and excessive sample disturbance when ascending

TABLE B7-1. SUMMARY OF BOTTOM SAMPLING EQUIPMENT[a]

Device	Use	Advantages	Disadvantages
Fluorocarbon plastic or Glass Tube	Shallow wadeable waters or deep waters if SCUBA available. Soft or semi-consolidated deposits.	Preserves layering and permits historical study of sediment deposition. Rapid–samples immediately ready for laboratory shipment. Minimal risk of contamination.	Small sample size requires repetitive sampling.
Hand Corer with removable Fluorocarbon plastic or glass liners	Same as above except more consolidated sediments can be obtained.	Handles provide for greater ease of substrate penetration. Above advantages.	Careful handling necessary to prevent spillage. Requires removal of liners before repetitive sampling. Slight risk of metal contamination from barrel and core cutter.
Box corer	Same as above.	Collection of large undisturbed sample allowing for subsampling.	Hard to handle.
Gravity corers, that is, Phleger Corer	Semi-consolidated sediments.	Low risk of sample contamination. Maintains sediment integrity relatively well.	Careful handling necessary to avoid sediment spillage. Small sample, requires repetitive operation and removal of liners. Time consuming.
Young Grab (fluorocarbon plastic- or kynar-lined modified 0.1 m^2 van Veen)	Lakes and marine areas.	Eliminates metal contamination. Reduced pressure wave.	Expensive. Requires winch.
Ekman or Box Dredge	Soft to semi-soft sediments. Can be used from boat, bridge, or pier in waters of various depths.	Obtains a larger sample than coring tubes. Can be subsampled through box lid.	Possible incomplete jaw closure and sample loss. Possible shock wave which may disturb the fines. Metal construction may introduce contaminants. Possible loss of "fines" on retrieval.
PONAR Grab Sampler	Useful on sand, silt, or clay.	Most universal grab sampler. Adequate on most substrates. Large sample obtained intact, permitting subsampling.	Shock wave from descent may disturb "fines". Possible incomplete closure of jaws results in sample loss. Possible contamination from metal frame construction. Sample must be further prepared for analysis.

TABLE B7-1. SUMMARY OF BOTTOM SAMPLING EQUIPMENT[a] (continued)

Device	Use	Advantages	Disadvantages
BMH-53 Piston Corer	Waters of 4-6 ft deep when used with extension rod. Soft to semi-consolidated deposits.	Piston provides for greater sample retention.	Cores must be extruded on site to other containers–metal barrels introduce risk of metal contamination.
Van Veen	Useful on sand, silt, or clay.	Adequate on most substrates. Large sample obtained intact, permitting subsampling.	Shock wave from descent may disturb "fines". Possible incomplete closure of jaws results in sample loss. Possible contamination from metal frame construction. Sample must be further prepared for analysis.
BMH-60	Sampling moving waters from a fixed platform.	Streamlined configuration allows sampling where other devices could not achieve proper orientation.	Possible contamination from metal construction. Subsampling difficult. Not effective for sampling fine sediments.
Petersen Grab Sampler	Useful on most substrates.	Large sample; can penetrate most substrates.	Heavy, may require winch. No cover lid to permit subsampling. All other disadvantages of Ekman and Ponar.
Shipek Grab Sampler	Used primarily in marine waters and large inland lakes and reservoirs.	Sample bucket may be opened to permit subsampling. Retains fine grained sediments effectively.	Possible contamination from metal construction. Heavy, may require winch.
Orange-Peel Grab Smith-McIntyre Grab	Useful on most substrates.	Designed for sampling hard substrates.	Loss of fines. Heavy–may require winch. Possible metal contamination.
Scoops, Drag Buckets	Various environments depending on depth and substrate.	Inexpensive, easy to handle.	Loss of fines on retrieval through water column.

[a] Comments represent subjective evaluations.

SOURCE: American Society for Testing and Materials, 1991

- allow easy access to the sample surface in order that undisturbed subsamples may be taken

Penetration well below the desired sampling depth is preferred to prevent sample disturbance as the device closes. It is optimal to use a sampler that has the means of weight adjustment in order that penetration depths may be modified.

Trawls and Dredges – Trawls and dredges generally collect organisms over a variable and relatively large area (Fredette *et al.*, 1989; U.S. EPA, 1986-1991). Their design and use precludes the collection of infaunal organisms at sediment depths greater than a few centimeters.

The data collected using these devices are qualitative since consistent and reproducible sampling of a constant area of the bottom is not possible. However, these devices are suitable for reconnaissance of potential sampling sites. Data collected using trawls or dredges may be used to generate a list of organisms present in the area. A list of species may be used to select sampling locations for a monitoring program.

Grab Samplers – Grab samplers are capable of consistent sampling of bottom habitats; depending on the size of the device, areas 0.02 to 0.5 m^2 and depths ranging from 5 to 15 cm may be sampled. Limitations of grab samplers include:

- variability among samples in penetration depth depending on sediment properties
- oblique angles of penetration which result in varying penetration depths within a sample
- the sample is inevitably folded resulting in the loss of information concerning the vertical structure of benthic communities in the sediments

However, the careful use of these devices will provide quantitative data. Grab samplers are the tools of choice for a number of estuarine and marine monitoring programs due to their ability to provide quantitative data at a relatively low cost (Fredette *et al.*, 1989; U.S. EPA, 1986-1991).

Core Samplers – Core samplers utilize a surrounding frame to ensure vertical entry; vertical sectioning of the sample is possible (U.S. EPA, 1986-1991). These devices are capable of maximum penetration depths of 15 cm and may collect volumes 5 to 10 times that of grab samplers. Limitations of box corers include:

- its large size and weight require the use of cranes or winches and a large vessel for deployment
- higher construction expenses
- lack of calibration studies to permit comparisons to grab samples

The Hessler-Sandia box corer uses dividers to section the core into subsections, facilitating subsampling of the core. Box corers are recognized as the tools of choice for maximum accuracy and precision when sampling soft-bottom habitats.

Suction Samplers – *In situ* suction devices use the flow of water to collect sediments and benthic organisms (Eleftheriou and Holme, 1984). An open-ended suction tube draws sediments and organisms into the flow of water; organisms are trapped in a sieve or mesh bag at the end of the tube. Limitations of suction devices include:

- little control of the volume of sediment collected
- collection of animals from surrounding sediments, inflating infauna abundances
- organisms are typically turbulently abraded during collection, making identifications even more difficult

Fleischack *et al.* (1985) propose modifications to suction samplers that overcome some of these limitations. Some samplers can be operated remotely; more often they are operated by divers. Any diver-operated collection procedures will be restricted to SCUBA depths and conditions permitting safe diving (e.g., relatively calm waters, reasonable visibility) thereby limiting their use in monitoring programs.

Sediment Profiling Camera – The sediment profiling camera is a unique tool which allows vertical, *in situ* imaging of the water-sediment interface. Photographs of biological activity may be made at a maximum depth of 18 cm, however, penetration depth of the viewing prism may be limited due to the physical characteristics of the sediment (i.e., penetration depths are greater in silt than sand).

The sediment profiling camera system can give measures of organism tube density, thickness of the pelleted layer, and infauna present in the image area (Rhoads and Germano, 1982). Although this tool provides qualitative information concerning the

benthos activity, the sediment profiling camera system cannot provide quantitative information on species diversity, abundance, and biomass. Information concerning species composition cannot be gained using this tool. Consequently, characterizations of the benthic community structure cannot be made from the data taken from these images.

The use of the sediment profiling camera is not recommended for the collection of benthic community structure data. However, the sediment profile camera proves to be effective as a reconnaissance tool. Delineation of physically similar sampling sites may be determined through the use of this tool, aiding in the selection of sampling stations. Ground-truthing of these images by means of laboratory analyses of collected material is highly recommended.

Recommendations – If quantitative analyses of benthic community structure are required, a grab or core sampler is recommended. Either of these sampling devices permit adequate sampling of both sediment and benthic infaunal communities with the one sampling device.

Area Sampled

Different species of benthic macroinvertebrates have different scales of horizontal and vertical spatial distribution (Elliot, 1971; Livingston *et al.*, 1976; Downing, 1979). Costs of laboratory analyses of the sample increase with increased sample unit size. Analyses of spatial and temporal scale, statistical power, as well as costs, will assist in determining optimal sample unit size. It is highly recommended that a standard sample unit size (same surface area and depth) be analyzed in order to ensure data comparability.

Sorting Protocols

There are several options which may be considered when designing sorting protocols. In order to assure comparability among studies, a consistent set of sorting procedures is highly recommended.

Sieving: Mesh Size and Location – The use of different sieve mesh sizes limits the comparability of results among estuarine monitoring studies (Reish, 1959; Rees, 1984). The major advantage of using a smaller mesh size is the retention of both juveniles and adults organisms as well as large- and small-bodied taxa. The major disadvantage is the concomitant increased cost of sample processing. For example,

using a 0.5 mm mesh over a 1.0 mm mesh could increase retention of total macrofaunal organisms by 130-180 percent; however, increased costs for processing the samples may be as much as 200 percent (U.S. EPA, 1986-1991).

It is recommended that a standard mesh size be selected for all monitoring studies within the estuary. In Puget Sound, the 1.0 mm mesh size is commonly used in impact assessments and the 0.5 mm mesh size is sometimes used in recruitment studies. The issue of data comparability was resolved by recommending sequential screening and analysis procedures (Becker and Armstrong, 1988). By screening samples through both the 1.0 and 0.5 mm sieves, the 1.0 mm fraction could be analyzed separately and the data compared with other impact studies throughout the estuary. The data for the 0.5 mm fraction of the samples could then be combined to yield information on the complete sample for comparison with other recruitment data.

Sieving can be conducted either aboard the survey vessel or onshore after the cruise is completed. The major difference is that aboard the vessel, sieving occurs prior to fixation; onshore, sieving occurs after fixation. Fixation of organisms in the sediment before being sieved has the problem of the timely distribution of adequate concentrations of the fixative to all the organisms in the sample. Sieving characteristics of organisms can be greatly modified by decomposition and deterioration of body parts. When a large number of samples are to be collected during a cruise, field sieving results in a significant reduction in the volume of material stored and later transported to the laboratory.

Use of Relaxants – The use of relaxants prior to sieving and fixation reduces the tendency of organisms to distort their shape or autotomize, and consequently facilitates taxonomic identifications and morphometric measurements (U.S. EPA, 1986-1991). Complete organisms, having a "natural" appearance, are easier to identify to lower taxonomic groups than are distorted or fragmented specimens.

The magnitude to which relaxants can influence taxonomic identification, thereby limiting comparisons among monitoring studies, is unknown. It is not recommended that comparisons be conducted between studies in which relaxants were used and those in which they were not used.

Use of Stains – Vital stains — primarily rose bengal — are added to samples to facilitate sorting. The stain colors most infauna, enhancing their contrast with background debris. Not all taxa stain adequately and some taxonomists have found that staining interferes with the identification of certain taxa. Although staining may aid

the sorting process, a proper quality control program should ensure that sorting efficiency is maintained whether or not a stain is used.

Level of Taxonomy

The necessity to identify organisms to the level of species has recently been questioned (Warwick, 1986). The liabilities of identifying organisms to the species level include:

- inaccurate and imprecise identifications (Ellis, 1985)
- identifications are time-consuming and costly; costs can be reduced as much as 30-50 percent by limiting identification of samples to higher taxonomic levels (U.S. EPA, 1986-1991)

Identifications to higher taxonomic groups can provide gross characterizations of benthic infaunal assemblages and may be sufficient to meet program objectives concerning detection of community responses to anthropogenic disturbance. Warwick (1986) contends that detection of anthropogenic disturbances would be facilitated since these perturbations impact community structure at taxonomic levels higher than the species level. Natural disturbances, which generally affect infauna community structure by species replacement, would have little influence on analyses at higher taxonomic levels.

However, identification to higher taxonomic levels may sacrifice the potential wealth of information available using species-level identification, such as analyses of population dynamics and productivity. Furthermore, it is generally regarded that the species is the taxon most responsive to environmental stress; individual species will tend to be replaced by another species within the same genus before the genus is replaced (U.S. EPA, 1986-1991).

The level of taxonomic identification will depend on monitoring program objectives, sample size, study sites, and analytical measures. Data based on lower taxonomic levels can be grouped for future comparisons with higher level taxa. It is strongly recommended that all samples identified only to higher taxonomic levels be properly archived, since comparisons of lower taxonomic data may be required later.

Selection of Sampling Period

Benthic infauna assemblages are dynamic; the most common temporal patterns observed in benthic communities are those associated with seasonal changes. Seasonal

variation in benthic assemblages may be due to changes in physical, chemical, and/or biological parameters: (e.g., temperature, light transmissivity, dissolved oxygen, predation, recruitment).

Given the seasonal variation characteristic of benthic assemblages in general, it is recommended that direct comparisons between samples collected during different seasons be avoided. Studies investigating interannual variation in the characteristics of benthic communities should be conducted during the same season (preferably the same month) each year.

B7.3 Existing Analytical Methods

There are a variety of approaches to assess the effects of anthropogenic perturbance on benthic communities of the estuary. Many assessment approaches are derived from modifications of Connell's (1978) Intermediate Disturbance Hypothesis. Some researchers have proposed that selected community structure measures — e.g., species composition, abundance, etc. — are predictable along a gradient of environmental disturbance (Pearson and Rosenberg, 1978; Gray and Mirza, 1979; Warwick, 1986). A corollary to this supposition is that indicator species, whose responses would epitomize community responses to habitat perturbations may be identified.

These assessment approaches may be grouped into three categories:

- biological indices
- indicator species
- multivariate analyses

However, there has been little consensus among biologists regarding the suitability of various techniques for describing community characteristics and/or for assessing estuarine impacts. A critical evaluation of the use of biological indices to detect environmental change is presented in an EPA Technical Support Document (U.S. EPA, 1985; Table B7-2). In addition to measures of changes in the abundances of pollution sensitive, pollution tolerant, and opportunistic species, the indices shown in Table B7-2 are evaluated on the basis of the following criteria:

- biological meaning
- ease of interpretation

TABLE B7-2. BIOLOGICAL INDICES

Index/Method	Biological Characteristic Measured	Recommended for Monitoring[a]
Biological integrity	Community structure	B
Bray-Curtis	Dissimilarity	P,B,F
Dominance[b]	Community structure	P,B,F
Infaunal index	Community structure	B[c]
No. individuals	Total abundance	P,B,F
No. species	Total taxa	P,B,F
Opportunistic and pollution tolerant species	Community structure	P,B
Pollution-sensitive species	Community structure	P,B
Biomass	Standing crop	P,B,F
Margalef's SR	Diversity	P,B,F
Pielou's J	Evenness	P,B,F[d]
Shannon-Wiener H'	Diversity	P,B,F[d]

[a]P (plankton), B (benthos), and F (fishes) indicate those biological groups to which a given index may be applied.

[b]Defined as the minimum number of species required to account for 75 percent of the individuals in a sample (see Swartz *et al.*, 1985).

[c]Where developed.

[d]May be used together as additional indices of community structure.

- sensitivity to community changes due to anthropogenic sources

The results of these evaluations and additional information on other analytical methods are summarized below.

Biological Indices

The numbers of individuals and the numbers of species have been found to be good indicators of anthropogenic disturbance, as well as of other environmental stresses (Table B7-2; Pearson and Rosenberg, 1978; Warwick, 1986). Furthermore, these simple biological indices are less ambiguous and are often as informative as diversity indices (U.S. EPA, 1985; Green, 1979; Hurlbert, 1971). Measures of biomass have inherent problems in the collection of the data — e.g., loss or gain of weight due to preservative medium, drying times, evaporative weight loss, etc. However, biomass, used in conjunction with species composition and abundance data, may demonstrate the relative effects of enrichment on benthic communities (Pearson and Rosenberg, 1978).

More complicated indices – e.g., biological integrity, species diversity, species richness, dominance, evenness – have found varying degrees of acceptance. Indices of diversity and biological integrity often have the following limitations (Green, 1979):

- often lack biological meaning
- are not robust empirical indicators of any important correlates of "estuarine health"
- do not incorporate information of form and function of resident species
- are susceptible to biases associated with well-described taxa

However, these indices are a widely used measure of community structure.

The dominance index is a measure of the degree to which one or a few species dominate the community. The dominance index, herein defined as the minimum number of species required to account for 75 percent of the total number of individuals, has been useful in describing community structure (Swartz *et al.*, 1985). It is easily calculated, does not assume an underlying distribution of individuals among species, and is statistically testable.

Several graphical analyses of these simple biological indices have been developed; most require further study before their effectiveness is known. For example, the use of Gray and Mirza's (1979) log-normal distribution of individuals per species and Warwick's (1985) abundance-biomass comparisons (ABC) methods, though useful for some habitats, have been found to be unpredictable for other habitats and other locales (U.S. EPA, 1989; Beukema, 1988).

Pearson and Rosenberg's (1978) species-abundance-biomass (SAB) model appears to be applicable to several forms of environmental disturbance and may be used in conjunction with other analyses to assess spatial and temporal impacts due to disturbance (Figure B7-1). The SAB model provides a means to assess the condition of the habitat based on species numbers, total abundance, and total biomass (Figure B7-1). Severely polluted areas are identified by the lack of organisms; low species numbers,

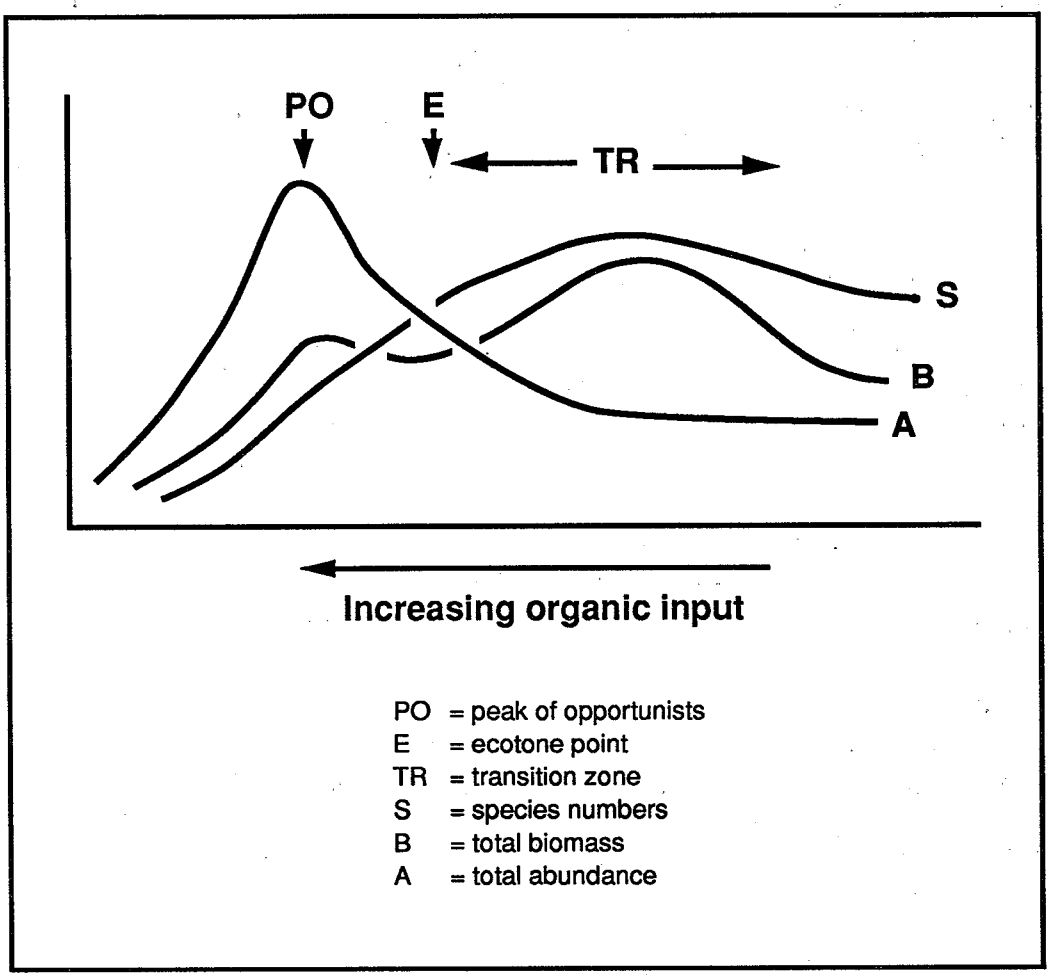

Figure B7-1. Generalized Species-Abundance-Biomass diagram of changes along a gradient of organic enrichment (from Pearson and Rosenburg, 1978).

total abundance, and biomass. Highly polluted areas are identified by a few highly-abundant populations of small-bodied, pollution-tolerant, opportunistic species (e.g., *Capitella capitata*); total abundance is high, although biomass and species numbers remain low. Less polluted areas (e.g., further from a point source, or over time following the cessation of organic input) may be identified by higher species numbers and biomass, as more competitive larger-bodied species are able to tolerate sediment conditions. More pristine areas are characterized by a number of small populations of competitively dominant species; species numbers remain elevated, although biomass and total abundance are low. However, this approach requires the comparison of two or more sites along the pollution gradient.

Indicator Species

Examination of abundances of individual indicator species are generally informative and may reduce the cost of the analysis. The absence of pollution sensitive species and the enhancement of opportunistic and pollution tolerant species may assist in defining the spatial and temporal extent and magnitude of impacts. However, indicator variables must possess the following characteristics (Green, 1984):

- provide sufficiently precise and accurate appraisals of:
  - species of concern
  - anthropogenic disturbances to benthic communities
  - presence/absence or the magnitude of anthropogenic perturbance to the estuarine ecosystem

- cost-effective and a statistically reliable alternative to monitoring all critical benthic community measures of habitat perturbance

- appropriate for the spatial and temporal scale demanded by the study objectives

Pearson and Rosenberg (1978) demonstrated that along a gradient of organic enrichment, a predictable community of benthic infauna could be observed (Figure B7-2); communities consist of opportunistic, tolerant species in areas of severe pollution, giving way to less tolerant and more competitively dominant species further from severely polluted areas (Figure B7-2). This model of indicator species composition and distribution been found to be useful in assessing both natural and anthropogenic disturbances.

Several indices, derived from the distributions of pollution sensitive species and opportunistic disturbance-tolerant species, have been developed in order to evaluate impacts to infaunal benthic community structures: nematode/copepod ratio (Raffaelli and Mason, 1981), infaunal index (Word, 1978), organism-sediment index (Rhoads and Germano, 1986). The infaunal index was initially developed for the Southern California Bight, and utilizes the abundances of four functional groupings to describe community structure. Low values of the infaunal index indicate communities dominated by deposit feeding organisms that are considered to be more impacted than communities dominated by suspension feeders (high infaunal index values) (Word, 1978). This index has been successfully adapted for Puget Sound by Thom *et al.* (1979).

Polluted or disturbed conditions may be indicated by:

- high values of the nematode/copepod ratio
- low values (< 65) of the infaunal index

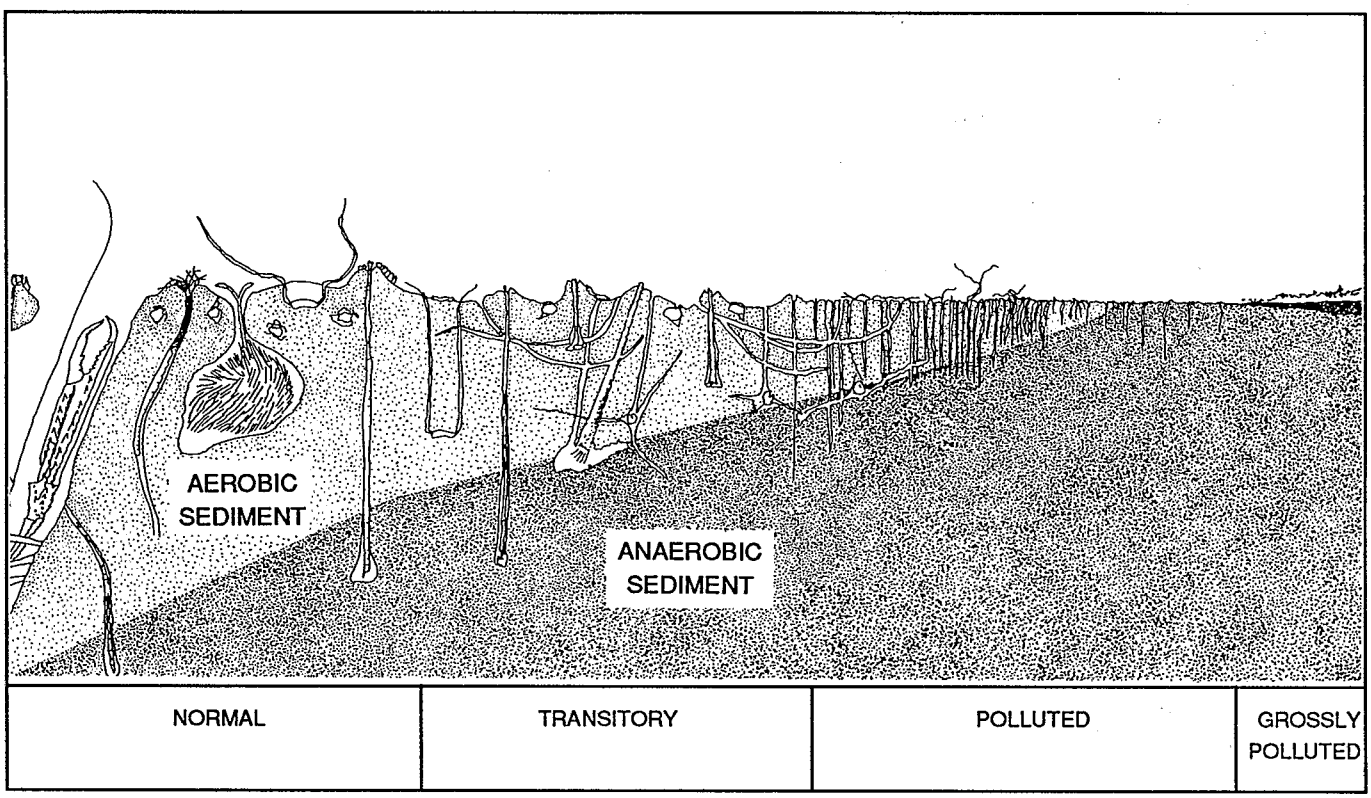

Figure B7-2. Diagram of changes in fauna and sediment structure along a gradient of organic enrichment (from Pearson and Rosenberg, 1978).

- low values (< +7) of the organism-sediment index

However, further studies of the response patterns of these indicator indices to anthropogenic perturbations are required in order to select appropriate indicators of benthic community impact.

It is recommended that multiple pollution-tolerant and pollution-sensitive indicator species are selected by clearly defined criteria. Multiple indicator species often provide a more complete representation of environmental conditions.

Multivariate Analyses

Numerical classification encompasses a wide variety of techniques that have been used in the analysis of benthic infauna data to distinguish groups of entities (e.g., sample locations) according to similarity of attributes (e.g., species). These techniques differ from most multivariate methods in that no assumptions are made concerning the underlying distributions of the variables. Detailed descriptions of numerical classification analysis can be found in Romesburg (1984), Clifford and Stephenson (1975), Boesch (1977), Sneath and Sokal (1973), and Anderberg (1973). Boesch (1977) is particularly valuable as an introduction and guide to the use of numerical classification analysis in marine environmental studies. Guidance on the interpretation of classification results is provided in U.S. EPA (1987, 1988).

Ordination analyses have also been used to reduce the dimensionality (i.e., the number of variables) of the data set while maintaining the relationship among similar and dissimilar entities. At present no single ordination technique has been shown to be clearly superior for the analysis of biological data (U.S. EPA, 1985).

Multivariate analyses are effective heuristic tools. They generate visual representations which often indicate where further analyses ought to be conducted.

Analytical Approach Recommendations

Some of the most informative measures of community structure are the simplest (Table B7-2):

- number of individuals

- number of species

- dominance
- infaunal index
- abundances of pollution sensitive species
- abundances of opportunistic and pollution tolerant species

These indices have proved to be useful over various habitats and regions in assessing changes to benthic community structures (U.S. EPA, 1985). Values for these indices may be determined from the list of species abundances generated during the taxonomic identifications of collected specimens. Furthermore, the values of these six variables may be easily tested statistically using parametric or nonparametric techniques. It is recommended that no single index or analytical method be used to assess impacts; rather the assessment of impacts should incorporate information that each variable and method contributes concerning benthic community structure.

Selection of Reference Sites

Selection of reference sites is key to the evaluation of environmental impact assessment. Monitoring of reference sites must provide measures of natural variability against which measures of anthropogenic impacts are detected. Thus, reference sites should be similar in every way with sampling sites of the monitoring program except that they are unaffected by anthropogenic disturbances of concern. Results of analyses using reference measures provide the means of discerning variability due to anthropogenic sources from natural variability of the ecosystem. Characteristics which should be controlled for include:

- sediment characteristics (i.e., grain size)
- water depth
- flow characteristics

Several reference sites may be required in order to meet these criteria.

B7.4 QA/QC Considerations

Sample Collection

Sampling device should be inspected for wear and tear potentially leading to sample leakage upon ascent. It is prudent to have a backup sampler on board the survey vessel in case the primary sampler is found to be unsuitable during the cruise.

The following sample acceptability criteria should be satisfied (U.S. EPA, 1987a):

- sediment is not extruded from the upper face of the sampler such that organisms may have been lost
- overlying water is present, indicating minimal leakage
- the sediment surface is relatively flat indicating minimal disturbance or winnowing (Figure B7-3)

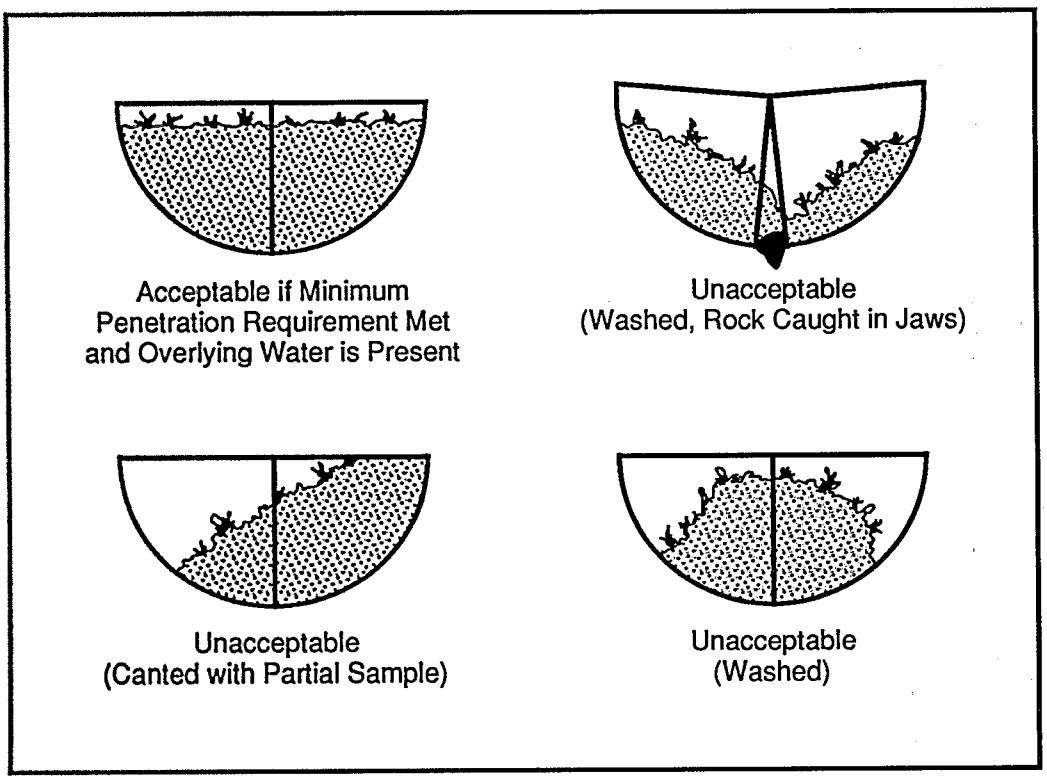

Figure B7-3. Examples of acceptable and unacceptable samples (U.S. EPA, 1986-1991).

- the entire surface of the sample is included in the sampler
- the desired penetration depth is achieved

If the sample does not meet all the criteria, the sample should be rejected.

Taxonomic Identification

A key QA/QC issue is taxonomic standardization. Consistent taxonomic identifications are achieved through interaction among taxonomists working on each major group. Participation of the laboratory staff in regional taxonomic standardization programs is recommended to ensure regional consistency and accuracy of identifications.

Five percent of all samples identified by one taxonomist should be re-identified by another taxonomist who is also qualified to identify organisms in that major group. It is advisable that at least three individuals of each taxon should be sent for verification to recognized experts. These verified specimens should then be placed in a permanent reference collection. All specimens in the reference collection should be stored in labeled vials which are segregated by species and sample. Reference specimens should be archived alphabetically within major taxonomic groups.

It is also recommended that at least 20 percent of each sample be re-sorted for QA/QC purposes. Re-sorting is the examination of a sample or subsample that has been sorted once and is considered free of organisms. Re-sorting should be done by someone other than the one who sorted the original sample. If a sample is found that does not meet the recommended 95 percent removal criterion, the entire sample should be re-sorted.

B7.5 Statistical Design Considerations

Consideration of statistical strategies will mitigate the high costs of collecting and processing samples. See also Statistical Design Considerations: Statistical Power and Power-Cost Analysis (Section B.3).

Temporal Stratification of the Data

The time of the year should be controlled or stratified in the design — the use of annual averages is seldom good practice. Temporal stratification of the data should not be attempted until sufficient knowledge of long-term natural cycles is attained. Initially, simple regression analyses may be conducted on seasonally stratified data in

order to identify monotonic temporal trends. Further examinations of whether conditions are improving or degrading over time may be examined using various statistical time series analyses.

B7.6 Use of Data

Monitoring of benthic community structure provides *in situ* measures of the benthic habitat and remains a powerful tool in the evaluation of spatial and temporal effects of anthropogenic and natural disturbances. The presence or absence of certain infaunal organisms are useful in indicating the previous condition of the environment. Monitoring of benthic infaunal communities also provides data required in the design and validation of benthic community dynamics models (e.g., Pearson and Rosenberg, 1978) and the selection of biological indicators (e.g., Word, 1978).

In addition, monitoring of benthic communities directly provides accurate information essential in assessing the effectiveness of pollution abatement programs (Bilyard, 1987). For example, benthic infauna monitoring provided information used to assess the effectiveness of pollution abatement plans in the recovery of Southern California waters (Reish, 1986; SCCWRP, 1988). These studies indicate that analyses of benthic infaunal communities may be effectively used to monitor long-term recovery of the receiving environment (Reish, 1986).

Currently, benthic communities have not proved useful for identifying specific chemicals or classes of chemicals present in the environment. Further information concerning specific responses to specific contaminants is required before infaunal community structure becomes useful in identifying sources of contaminants (U.S. EPA, 1989). In addition, the use of benthic community structure to predict specific effects on potential predators is cautioned. Information on trophic relationships, competition, and predation is often not available. The capability to predict the effects of altered prey communities on predators may improve with research on these topics. Factors such as prey quality, distribution of prey, and interactions among species will be important components of this research.

However, benthic invertebrates serve as effective indicators of environmental conditions; delineating the magnitude, spatial extent, and temporal trends of anthropogenic and natural perturbations to the ecosystem (Reish, 1986; Bilyard, 1987). Monitoring of benthic infauna will provide relevant and accurate data fundamental to achieving the objectives of most estuarine monitoring programs.

B7.7 Summary and Recommendations

Rationale

- The objective is to detect and describe spatial and temporal changes in the structure and function of benthic communities
- Provides *in situ* measures of habitat quality and is a powerful tool in assessing environmental impact

Monitoring Design Considerations

- It is recommended that consistent types of sampling gear, sample sorting protocols, level of taxonomy, and location and timing of sample collection be implemented to allow for comparisons among studies
- Collection of undisturbed sediment requires that the sampler:
  - create a minimal pressure wave when descending
  - form a leakproof seal when the sediment sample is taken
  - prevent winnowing and excessive sample disturbance when ascending
  - allow easy access to the sample surface in order that undisturbed subsamples may be taken
- Penetration well below the desired sampling depth is preferred to prevent sample disturbance as the device closes.
- Grab samplers and box corers are recognized as the tools of choice for maximum accuracy and precision when sampling soft-bottom habitats
- Sorting through a standard sieve mesh size is recommended – further sorting through other mesh sizes may be conducted in addition to sorting through this standard mesh size
- Relaxants facilitate identification and morphometric measurements, however standard procedures must be implemented to ensure comparability among studies
- Vital stains may facilitate sorting, however a proper QA program should ensure that sorting efficiency is maintained

- Identifications to higher taxonomic levels may be sufficient to meet program objectives, however it is recommended that all samples be archived since comparisons to lower taxonomic levels may be required at a later date

- In order to reduce the variation due to seasonal differences, sampling should be conducted during the same season – preferably the same month – each year

Existing Analytical Methods

- It is recommended that simple measures of community structure be used to assess the condition of the estuarine benthos: number of individuals, number of species, dominance, infaunal index, abundance of pollution sensitive species, and abundance of pollution tolerant species (Table B3-1)

- Selected biological indices should retain biological meaning, be robust indicators of estuarine "health", and incorporate species form and function

- Indicator species should possess the following characteristics:
  - sensitive to benthic perturbances of concern,
  - cost-effective and statistically reliable alternative to measuring all species in a monitoring program
  - statistically reliable indicative measures of habitat perturbance
  - appropriate for the spatial and temporal scale demanded by the study objectives

- Selection of reference sites is key to the evaluation of environmental impact due to anthropogenic perturbances; several reference sites may be required to provide proper control for sampling sites

QA/QC Considerations

- Taxonomic standardization is key to the analysis of community structure – recommended protocols include consistent interactions among taxonomists, re-identification of selected samples, use of a reference collection, and re-sorting of selected samples or subsamples

Statistical Design Considerations

- Power analyses may be applied to determine the appropriate number of sample replicates required to detect a specified difference, thereby optimizing the high costs of collecting and processing samples

Use of Data

- Provide essential information in order to assess impacts due to anthropogenic perturbance, monitor recovery of the receiving environment, and validate community and population models

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B8.0 Fish Community Structure

B8.1 Rationale

Fish are important economic, recreational, and aesthetic components of the estuarine ecosystem. To protect and preserve healthy fish stocks, reliable estimates of fish population abundances, a detailed knowledge of fish life histories, and information on the effects of natural and anthropogenic disturbance are required.

The objective of monitoring fish populations is to detect and describe spatial and temporal changes in the structure and function of fish communities. These results can be used to assess the condition – the "health" – of estuarine habitats, to monitor rates of recovery following environmental interventions, and to provide an early warning of potential impacts to recreational and commercial fisheries. In addition, certain demersal fish are good indicators of benthic conditions since:

- they are generally sedentary – observed effects are in response to local environmental conditions
- they are relatively sensitive to habitat disturbance – i.e., communities undergo changes in abundance in response to environmental perturbations
- they often mediate the transfer of nutrients and toxic substances in the ecosystem as important predator and prey organisms

Monitoring fish population abundances is one of a handful of methods which provide *in situ* measures of biotic health and habitat quality. The assessment of fish populations remains a powerful tool in the evaluation of the spatial and temporal effects of anthropogenic and natural disturbances.

B8.2 Monitoring Design Considerations

Collection, analyses, and evaluations of fish community structure and function are typically time-consuming, labor-intensive, and expensive tasks. A survey vessel manned by an experienced crew and specially equipped with gear to collect organisms is required. Expert taxonomists are needed to identify and enumerate collected fish specimens.

The results of fish monitoring programs can vary substantially depending on the objectives and corresponding design specifications. Monitoring design characteristics that may affect this variability include:

- type of sampling gear
- volume sampled

- location and timing of sample collection

It is essential to understand the effects of these monitoring design characteristics on the results and to standardize them as much as possible to ensure the comparability of data throughout the estuary.

Analyses of power-cost efficiencies are useful in selecting the appropriate sampling gear and sample processing protocols. Ferraro *et al.* (1989) provide an example of power-cost analyses.

Sampling Devices

Sample collection protocols influence all subsequent laboratory and data analysis; it is key that fish population samples be collected using acceptable and standardized techniques. Several types of devices can be used to collect fish samples: traps and cages, passive nets, trawls (active nets), and photographic surveys (Fredette *et al.*, 1989). Many of these devices selectively sample specific types of fish. Accordingly, conducting comparisons among data collected using different devices is inadvisable.

Traps and Cages – Traps and cages are usually designed to attract and capture specific organisms. They are useful in studies examining the activities of a particular target organism in a given area. Traps and cages provide only qualitative measures of organisms in a particular area.

Passive Nets and Trawls – Nets are highly selective in the species that are captured and in the efficiency of retaining captured specimens. The size of the net, its configuration and orientation, and the avoidance behavior of the target species should be considered when using any net. The mesh size effects the speed at which the net can be towed. The slower the towing speed, the more likely that some organisms will either avoid or escape the net. It is highly recommended that the duration, direction, and speed of towing be standardized in order to compare trawls.

Passive nets (e.g., gill nets) are deployed at a fixed position; organisms become entangled or trapped within the netted area. Passive nets are used to collect selected target species and provide a qualitative means of sampling fish populations. Limitations associated with passive nets include:

- nets, ordinarily, must remain in-place for an extended period of time

- deployment and recovery of nets are typically time-consuming processes

Trawls (active nets) are drawn through the water and results are more immediate compared to passive nets. Trawls are typically used to collect large quantities of fish at various depths (Fredette *et al.*, 1989). Otter trawls are less selective than passive nets, capturing a wider variety of fish and epibenthic macroinvertebrates.

Photographic Surveys – Photographic surveys are effective when the bottom topography is uneven or where trawling is not possible. However, the utility of photographic surveys is limited by water clarity, difficulties identifying species, and fish avoidance of the camera system. In addition, further studies comparing photographic surveys to trawls are required before comparisons may be made. This method is also limited by the qualitative nature of the data.

Volume Sampled

Different species of fish have different scales of horizontal and vertical spatial distribution (Cushing, 1975; Bond, 1979). Costs of laboratory analyses of the samples increase with increased volume sampled. Analyses of spatial and temporal scale, statistical power, as well as costs, will assist in determining optimal sample volume. It is highly recommended that a standard sample volume (same tow duration, tow speed, and net opening area) be analyzed in order to ensure data comparability.

Selection of Sampling Period

Fish assemblages are dynamic; the most common temporal patterns observed in fish communities are those associated with seasonal changes. Seasonal variation in fish assemblages may be due to changes in physical, chemical, and/or biological parameters: i.e., temperature, light transmissivity, dissolved oxygen, predation, recruitment, etc.

Given the seasonal variation characteristic of fish assemblages in general, it is recommended that direct comparisons between samples collected during different seasons be avoided. Studies investigating interannual variation in the characteristics of fish communities should be conducted during the same season (preferably the same month) each year.

B8.3 Analytical Methods Considerations

There are a variety of approaches to assess the effects of anthropogenic perturbance on fish communities of the estuary. These assessment approaches may be grouped into three categories:

- biological indices
- indicator species
- multivariate analyses

However, there has been little consensus among biologists regarding the suitability of various techniques for describing community characteristics and/or for assessing estuarine impacts. A critical evaluation of the use of biological indices to detect environmental change is presented in an EPA Technical Support Document (U.S. EPA, 1985; Table B8-1). In addition to measures of change in the abundance of pollution sensitive, pollution tolerant, and opportunistic species, the indices shown in Table B8-1 are useful. These indices are evaluated on the basis of the following criteria:

- biological meaning
- ease of interpretation
- sensitivity to community changes due to anthropogenic sources

The results of these evaluations and additional information on other analytical methods are summarized below.

Biological Indices

The numbers of individuals and the numbers of species have been found to be good indicators of anthropogenic disturbance, as well as of other environmental stresses (U.S. EPA, 1985). Furthermore, these simple biological indices are less ambiguous and are often as informative as diversity indices (U.S. EPA, 1985; Green, 1979; Hurlbert, 1971). Measures of biomass have inherent problems in the collection of the data – e.g., loss or gain of weight due to preservative medium, drying times, evaporative weight loss.

More complicated indices (e.g., species diversity, species richness, dominance, evenness) have found varying degrees of acceptance. Diversity indices, measures of the distribution of individuals among species, have the following limitations (Green, 1984):

- often lack biological meaning

TABLE B8-1. BIOLOGICAL INDICES

Index/Method	Biological Characteristic Measured	Recommended for Monitoring[a]
Biological integrity	Community structure	B
Bray-Curtis	Dissimilarity	P,B,F
Dominance[b]	Community structure	P,B,F
No. individuals	Total abundance	P,B,F
No. species	Total taxa	P,B,F
Opportunistic and pollution tolerant species	Community structure	P,B
Pollution-sensitive species	Community structure	P,B
Biomass	Standing crop	P,B,F
Margalef's SR	Diversity	P,B,F
Pielou's J	Evenness	P,B,F[c]
Shannon-Wiener H'	Diversity	P,B,F[c]

[a] P (plankton), B (benthos), and F (fishes) indicate those biological groups to which a given index may be applied.

[b] Defined as the minimum number of species required to account for 75 percent of the individuals in a sample (see Swartz *et al.*, 1985).

[c] May be used together as additional indices of community structure.

- are not robust empirical indicators of any important correlates of "estuarine health"

- do not incorporate information of form and function of resident species

- are susceptible to biases associated with well-described taxa

However, species diversity indices are a widely used measure of community structure.

The dominance index is a measure of the degree to which one or a few species dominate the community. The dominance index, herein defined as the minimum number of species required to account for 75 percent of the total number of individuals, has been useful in describing community structure (Swartz *et al.*, 1985). It is easily calculated, does not assume an underlying distribution of individuals among species, and is statistically testable.

Indicator Species

Examination of abundances of individual indicator species are generally informative and may reduce the cost of the analysis. The absence of pollution sensitive species and the enhancement of opportunistic and pollution tolerant species may assist in defining the spatial and temporal extent and magnitude of impacts. However, indicator variables must possess the following characteristics (Green, 1984):

- provide a sufficiently precise and accurate appraisals of:
  - specie(s) of concern
  - anthropogenic disturbances to benthic communities
  - presence/absence or the magnitude of anthropogenic perturbance to the estuarine ecosystem

- cost-effective and a statistically reliable alternative to monitoring all critical community measures of habitat perturbance

- appropriate for the spatial and temporal scale demanded by the study objectives

Further studies of the response patterns of fish species subjected to anthropogenic perturbations are required in order to select appropriate indicators of environmental impact. When a suitable indicator species is identified, it is desirable to monitor the

status and trend of that species' population. This will also be true of certain species having high economic or public value (e.g., striped bass). A wide variety of methods can be used to measure the size of fish populations and assess population structure. These include mark and recapture techniques and various techniques used to determine population sex and age structure. Nielsen and Johnson (1984) and Cailliet *et al.* (1986) provide thorough discussions of these techniques and their applications. Ricker (1975) provides an excellent discussion of methods for the sampling of fish populations.

Multivariate Analyses

Numerical classification encompasses a wide variety of techniques that have been used in the analysis of fish data to distinguish groups of entities (e.g., sample locations) according to similarity of attributes (e.g., species). These techniques differ from most multivariate methods in that no assumptions are made concerning the underlying distributions of the variables. Detailed descriptions of numerical classification analysis can be found in Romesburg (1984), Clifford and Stephenson (1975), Boesch (1977), Sneath and Sokal (1973), and Anderberg (1973). Boesch (1977) is particularly valuable as an introduction and guide to the use of numerical classification analysis in marine environmental studies. Guidance on the interpretation of classification results is provided in an EPA Technical Support Document (U.S. EPA, 1988).

Ordination analyses have also been used to reduce the dimensionality of the data set while maintaining the relationship among similar and dissimilar entities. At present no single ordination technique has been shown to be clearly superior for the analysis of biological data (U.S. EPA, 1985).

Multivariate analyses are effective heuristic tools. They generate visual representations which often indicate where further analyses ought to be conducted.

Analytical Approach Recommendations

Some of the most informative measures of community structure are the simplest (Table B8-1):

- number of individuals
- number of species
- dominance

- abundances of pollution sensitive species
- abundances of opportunistic and pollution tolerant species

These indices have proved to be useful over various habitats and regions in assessing changes to fish community structures (U.S. EPA, 1985). Values for these indices may be determined from the list of species abundances generated during the taxonomic identifications of collected specimens. Furthermore, the values of these six variables may be easily tested statistically using parametric or nonparametric techniques. It is recommended that no single index or analytical method be used to assess impacts; rather the assessment of impacts should incorporate information that each variable and method contributes concerning benthic community structure.

Selection of reference sites is key to the evaluation of environmental impact assessment.

Results of analyses using reference measures provide the means of comparison by which anthropogenic impacts are detected. It is essential that selected reference sites exhibit similar:

- bottom characteristics (i.e., bottom topography, sediment grain size, percent cover)
- water depths
- flow characteristics

compared to monitoring program sampling sites. Several reference sites may be required in order to meet these criteria.

B8.4 QA/QC Considerations

Sample Collection

The primary consideration for an adequate sampler is that it consistently collect undisturbed samples. Nets should be inspected for wear and tear potentially leading to sample loss. It is prudent to have backup gear on board the survey vessel in case the primary gear is found to be unsuitable or lost during the cruise.

Taxonomic Identification

A key QA/QC issue is taxonomic standardization. Consistent taxonomic identifications are achieved through interaction among taxonomists working on each major group. Participation of the laboratory staff in regional taxonomic standardization programs is recommended to ensure regional consistency and accuracy of identifications.

The verified specimens should then be placed in a permanent reference collection. All specimens in the reference collection should be stored in labeled vials which are segregated by species and sample. Reference specimens should be archived alphabetically within major taxonomic groups.

B8.5 Statistical Design Considerations

Consideration of statistical strategies will mitigate the high costs of collecting and processing samples. See also Statistical Design Considerations: Statistical Power and Power-Cost Analysis (Appendix B Introduction; Section B.3).

Temporal Stratification of the Data

The time of the year should be controlled or stratified in the design; the use of annual averages is seldom good practice, especially for species which are seasonally abundant. Temporal stratification of the data should not be attempted until sufficient knowledge of long-term natural cycles is attained. Initially, simple regression analyses may be conducted on seasonally stratified data in order to identify monotonic temporal trends. Further examinations of whether conditions are improving or degrading over time may be examined using various statistical time series analyses (e.g., temporal autocorrelation, spectral analyses).

B8.6 Use of Data

Monitoring of fish community structure provides *in situ* measures of the estuarine habitat and remains a powerful tool in the evaluation of spatial and temporal effects of anthropogenic and natural disturbances. The presence or absence of certain fish species is useful in indicating the condition of the environment. Monitoring of fish communities also provides data required in the design and validation of fish population dynamics models and the selection of biological indicators (U.S. EPA, 1990). Fishing mortality, both commercial and sport, is an important source of anthropogenic impact on fish populations. Before the effects of pollution can be assessed, fishing impacts must be considered. There are several methods available to assess the effects of fishing mortality, allowing pollution impacts to be determined (Ricker, 1975).

In addition, monitoring of fish communities may directly provide accurate information essential in assessing the effectiveness of pollution abatement programs. Analyses of fish communities may be effectively used to monitor long-term recovery of the receiving environment (Cushing, 1975).

Fish serve as effective indicators of environmental conditions; delineating the magnitude, spatial extent, and temporal trends of anthropogenic and natural perturbations to the ecosystem. Monitoring of fish populations will provide relevant data fundamental to achieving the objectives of many estuarine monitoring programs.

B8.7 Summary and Recommendations

Rationale

- The objective is to detect and describe spatial and temporal changes in the structure and function of fish communities in order to protect the economic, recreational, and aesthetic value of the estuarine fisheries
- Provides *in situ* measures of habitat quality and is a powerful tool in assessing environmental impact

Monitoring Design Considerations

- It is recommended that consistent types of sampling gear, volume sampled, and location and timing of sample collection be implemented to allow for comparisons among studies
- In order to reduce the variation due to seasonal differences, sampling should be conducted during the same season – preferably the same month – each year

Existing Analytical Methods

- It is recommended that simple measures of community structure be used to assess the condition of the estuarine fish: number of individuals, number of species, dominance, abundance of pollution sensitive species, and abundance of pollution tolerant species (Table B8-1)
- Selected biological indices should retain biological meaning, be robust indicators of estuarine "health", and incorporate species form and function

- Indicator species should possess the following characteristics:
  - sensitive to perturbances of concern
  - cost-effective and statistically reliable alternative to measuring all species in a monitoring program
  - statistically reliable indicative measures of habitat perturbance
  - appropriate for the spatial and temporal scale demanded by the study objectives

- Selection of reference sites is key to the evaluation of environmental impact due to anthropogenic perturbances; several reference sites may be required to properly control for sampling sites

QA/QC Considerations

- Taxonomic standardization is key to the analysis of community structure -- recommended protocols include consistent interactions among taxonomists, re-identification of selected samples, and use of a reference collection

Statistical Design Considerations

- Power analyses may be applied to determine the appropriate number of sample replicates required to detect a specified difference, thereby optimizing the high costs of collecting and processing samples

Use of Data

- Provide essential information in order to assess impacts due to anthropogenic perturbance, monitor recovery of the receiving environment, and validate community and population models

B8.8 Literature Cited and References

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B9.0 Fish and Shellfish Pathobiology

Pathobiological methods provide information concerning damage to organ systems of fish and shellfish through an evaluation of their structure, activity, and function. **Anatomic pathology** methods can give an indication of the nature of an altered state, for example, by identifying the specific type of tumor present in an animal. **Reproductive developmental** studies examine the reproductive capacity of animals and can provide information to aid in estimating and predicting population abundance and recruitment. **Biochemical/enzymological** studies seek to detect differences in enzymatic activity as a measure of biological condition. **Immunological** methods can demonstrate altered immune response, an indicator of changes in bodily defense mechanisms and susceptibility to disease.

Pathobiological methods should be used in concert to investigate cause and effect relationships as a result of contaminant exposure. Anatomic pathology can serve as a vital link between observed effects on populations and communities in an estuary and the changes in activity and function observed by other methods.

B9.1 Rationale

Pathobiological methods can be used to examine adverse effects of pollutants on fish and shellfish. The presence of toxics in water and sediments may not immediately result in visible changes in these organisms. Biomarkers offer a more sensitive and reliable assessment of exposure risks than ambient water or sediment quality monitoring. Monitoring of pathobiological effects provide information necessary to make determinations of the existence of adverse effects in animals (e.g., tumors), population productivity and stability (affected by reproduction and disease states) and the loss of organisms deemed valuable for ecological, aesthetic, recreational, scientific or economic reasons. Table B9-1 outlines some of the terms used to describe pathobiological methods.

Although the value of these methods for establishing cause and effect links has been established during laboratory toxicity studies, some questions remain regarding their ability to establish such links for field-collected organisms that are exposed to a variety of natural environmental stresses and combinations of contaminants (Hinton and Couch, 1984; Couch and Harshbarger, 1985; Mix, 1986; Sindermann, 1991). However, properly conducted multidisciplinary monitoring studies using these methods can provide regulatory agencies with evidence of impaired health status in animals exposed to contaminants in estuarine ecosystems. This information can then be used to direct laboratory confirmation of the cause, if necessary (see for example, Buckley *et al.*, 1985; Gardner *et al.*, 1991). Continued monitoring with these methods can be used to detect changes in a population's health during and following environmental interventions. Because changes at the organismal level precede changes in population

TABLE B9-1. LIST OF PATHOBIOLOGICAL TERMS

biomarker	=	any biological method used to detect the exposure of organisms to hazardous chemicals in the environment by measuring the response of the organisms to the contaminant through comparative molecular, biochemical, physiological, or anatomical observations of cellular dysfunction
cytochrome P450	=	a protein in the microsomes of liver cells which is important in catalyzing the metabolism of steroid hormones and fatty acids and in the detoxification of a variety of chemical substances
cytogenetics	=	the study of cytology in relation to genetics, especially the study of chromosomal behavior in mitosis and meiosis. Modern cytogenetics has led to the identification of chromosomes as bearers of the genes and deoxyribonucleic acid (DNA) as the key molecule of the gene
genotoxic agents	=	chemical and physical agents that can produce genetic alterations at subtoxic concentrations and which can result in altered heredity characteristics. Genotoxic agents generally possess specified chemical or physical properties that facilitate their interaction with nucleic acids
hepatic	=	of, or relating to, the liver
histopathology	=	pathologic histology; the science or study dealing with the cytologic and histologic (microscopic) structure of abnormal or diseases cells, tissues, and organs in relation to their function
histochemistry	=	the study of the chemistry of cells and tissues using light and electron microscopy, special chemical tests and special stains to determine the location of certain enzyme systems or reaction products in the cell
immunoassay	=	measuring the protein and protein-bound molecules that are concerned with the reaction of an antigen with its specific antibody, as in the detection of hormones or other substances
immunology	=	the study of being protected from a disease; the study of the response of the body and its tissues to a variety of antigens, including red cells, pollens, transplanted tissues and even the individual's own cells
inclusion bodies	=	bodies present in the nucleus or cytoplasm of certain cells in cases of infection by filtrable viruses or as the result of degenerative diseases or exposure to chemicals
macrophage	=	cells of the reticuloendothelial system having the ability to phagocytose particulate substances and to store vital dyes and other colloidal substances
pathobiology	=	pathology, the study of disease, with emphasis more on the biological than on the medical aspects of the essential nature, causes, and development of abnormal conditions, as well as the structural and functional changes that result from the disease process
smooth endoplasmic reticulum (SER)	=	a connecting network of tubules that course through the cytoplasm of the cell and which can be viewed using the electron microscope and is essential to metabolic functions of the cells

(Stedman's Medical Dictionary, 1982; Taber's Cyclopedic Medical Dictionary, 1985)

and community characteristics, pathobiological studies can provide an early indication of the effectiveness of management actions.

B9.2 Monitoring Design Considerations

A field survey to collect target organisms and tissue samples may be required for pathobiological monitoring (Hargis *et al.*, 1984; U.S. EPA, 1987a). In certain instances, a large sample size may be needed to establish statistical significance because of normal variation from animal to animal, species and generic differences, and migratory habits of fish. For these reasons, and the labor-intensive nature of pathobiological methods, the availability and allocation of funds, time, equipment, and trained personnel must be considered when planning to include pathobiological methods in monitoring programs.

Selection of Target (Indicator) Species

A key component of any pathobiological monitoring program is the selection of target species. The fundamental criterion is the ability to use the selected species to make comparisons between sampling locations and sampling periods.

It is recommended that the target species possess the following characteristics:

- abundant enough, temporally and spatially, to allow for adequate sampling
- large enough to provide adequate amounts of tissue for analysis
- sedentary (non-migratory) in nature to assure that pathobiological abnormalities are representative of the study area
- easily collected
- existing database of exposures and sensitivities

U.S. EPA has provided a list of suggested fish target species for different regions in the United States (U.S. EPA, 1987a; Table B9-2). Fish provide sufficient tissue for analyses and may indicate potential threats to human populations. However, fish are motile and pathobiological abnormalities detected may not be representative of the study area.

Bivalve molluscs, either attached to the substratum or burrowing in sediments, have been sampled extensively to examine the condition of these commercially important

TABLE B9-2. HIGHEST RANKING CANDIDATE FISHES FOR USE AS PATHOBIOLOGY MONITORING SPECIES

State	Locality	Species	Secondary Selection Criteria: Economic Importance	Secondary Selection Criteria: Bioassay Species
MASSACHUSETTS	Swampscott	Winter flounder	Yes	Yes
		Yellowtail flounder	Yes	Yes
		Ocean pout	No	No
		Windowpane	No	No
	Lynn	Winter flounder	Yes	Yes
		Yellowtail flounder	Yes	No
		Ocean pout	No	No
	South Essex	Winter flounder	Yes	Yes
		Yellowtail flounder	Yes	No
		Windowpane	No	No
		American eel	No	No
		Ocean pout	No	No
	Boston	Winter flounder	Yes	Yes
		Yellowtail flounder	Yes	No
		Ocean pout	No	No
		Windowpane	No	No
	Fall River	Winter flounder	Yes	Yes
		Windowpane	No	No
	New Bedford	Winter flounder	Yes	Yes
		Scup	Yes	No
		Summer flounder	Yes	Yes
RHODE ISLAND	Newport	Winter flounder	Yes	Yes
		Scup	Yes	No
		Weakfish	Yes	No
NEW YORK	Upper East River	Winter flounder	Yes	Yes
		Windowpane	No	No
		Weakfish	Yes	No
	Lower East River	Spot	Yes	Yes
		Scup	Yes	No
		American eel	No	No
	Lower Hudson River	Hogchoker	No	No
NEW JERSEY	Cape May	Spot	Yes	Yes
		Red hake	No	No
		Windowpane	No	No
		Summer flounder	Yes	Yes
VIRGINIA	Portsmouth	Spot	Yes	Yes
		Summer flounder	Yes	Yes
		Atlantic croaker	Yes	No
		Hogchoker	No	No
	Virginia Beach	Spot	Yes	Yes
		Red hake	No	No
		Summer flounder	Yes	Yes

TABLE B9-2.
(continued)

State	Locality	Species	Secondary Selection Criteria: Economic Importance	Secondary Selection Criteria: Bioassay Species
CALIFORNIA (NORTHERN)	San Francisco	English sole	Yes	Yes
		Pacific sanddab	Yes	No
		Big skate	No	No
	Oakland	English sole	Yes	Yes
		Starry flounder	Yes	No
		Pacific staghorn sculpin	No	No
	Monterey	English sole	Yes	Yes
		Curlfin sole	Yes	No
	Santa Cruz	English sole	Yes	Yes
	Watsonville	English sole	Yes	Yes
		Curlfin sole	Yes	No
CALIFORNIA (SOUTHERN)	Goleta	Dover sole	Yes	No
		Pacific sanddab	Yes	No
		Longspine combfish	No	No
		Spotted cusk-eel	No	No
	Santa Barbara	English sole	Yes	Yes
		Pacific sanddab	Yes	No
	L.A. County	Dover sole	Yes	No
		Curlfin sole	Yes	No
		English sole	Yes	Yes
	Orange County	Dover sole	Yes	Yes
		Pacific sanddab	Yes	Yes
		English sole	Yes	Yes
	Hyperion	Dover sole	Yes	No
	Oceanside	Longspine combfish	No	No
		Big skate	No	No
	Escondido	California skate	No	No
	San Elijo	Dover sole	Yes	No
		Blackbelly eelpout	No	No
		Pacific sanddab	Yes	No
		English sole	Yes	Yes
	San Diego	Dover sole	Yes	No
		English sole	Yes	Yes
		Pacific sanddab	Yes	No
		Longspine combfish	No	No
WASHINGTON	Central Puget Sound	English sole	Yes	Yes
		Dover sole	Yes	No
		Rock sole	Yes	No
		Spotted ratfish	No	No
		Rex sole	Yes	No
		C-O sole	Yes	No

estuarine species (i.e., Mussel Watch, National Status and Trends program). The most common target species have been oysters (*Crassostrea virginica*) and mussels (*Mytilus* spp.), although pathobiological and bioassay studies have also been performed on other species, such as crabs and penaeid shrimp (see also Table B10-3).

Secondary considerations, based on economic importance and status as a bioassay organism, may be applied to further winnow the list of candidate target species to a practical number of species to be analyzed.

Sampling Location

Appropriate locations of sampling stations depend on the objectives of the study. For example, to evaluate whether there is a statistically significant increase in lesions, stations should be located to collect specimens from contaminated and uncontaminated (background or control) areas for statistical comparison. It is also important to demonstrate a dose-response relationship between the pollutant concentration and tumor incidence by locating sample stations along a contamination gradient (i.e., from highly contaminated to moderately contaminated to uncontaminated). It is recommended that stations be located in areas where the geographic area of contamination is large enough that sampled fish could reasonably be expected to have spent a considerable amount of time within the influence of the pollutant (U.S. EPA, 1987a). Nielsen and Johnson (1984) and Cailliet *et al.* (1986) may be consulted for further fish sampling methodologies. Information on sampling bivalves and other invertebrates is contained in Couch (1978), Yevich and Barszcz (1983), Turgeon *et al.* (1991), and other sources.

B9.3 Existing Analytical Methods

Anatomic Pathology Methods

Anatomic pathology methods examine tissues with the naked eye (gross anatomic pathology), the aid of a light microscope (LM methods), or the electron microscope (EM methods). Gross anatomic methods are concerned with obvious adverse changes in tissue which can be observed in the field (Hunn, 1988; Hargis *et al.*, 1984). The advantage of gross methods is that large numbers of specimens can be examined rapidly. However, the methods are generally nonspecific (i.e., it is not possible to determine that a specific pollutant led to a specific disease). An exception to this is cataracts in fish, which have been linked with polynuclear aromatic hydrocarbon pollution in the field (Hargis and Zwerner, 1988).

A disadvantage of anatomic pathology methods is that they require specialized personnel and laboratories. The methods are, however, generally standardized, routine, and

operational in existing federal, state, university, veterinary, and private diagnostic laboratories that specialize in aquatic animal pathology. Current activities are aimed at developing a field-to-laboratory response-diagnostic scenario where adverse effects are found in the field, diagnosis is made in the laboratory, and experimental studies are initiated to verify results (May, 1990). See Yevich and Barszcz (1980), Howard and Smith (1983), Johnson and Bergman (1984), Klontz (1985), Meyers and Hendricks (1985), and U.S. EPA (1987a) for more information on these methods and techniques.

Light Microscopy Methods – LM (histologic) methods use the light microscope to view cells and tissues. These methods can detect changes such as inflammatory responses, alterations in the appearance of cells, cancerous/precancerous lesions, and damage due to parasites (U.S. EPA, 1986). The advantages of LM methods are that they are organ specific (i.e., the lesion can be localized to a specific organ) and can detect microscopic and subtle cellular alterations that are not evident on visual inspection. However, LM methods are more expensive (approximately $30/sample) and slower than gross methods (May, 1990).

Electron Microscopy Methods – EM methods use the electron microscope to detect changes in tissue at cellular and subcellular levels, such as the identification of the nature of inclusion bodies or changes in the amount of smooth endoplasmic reticulum. An advantage of EM methods is that they can be highly specific because the pollutant can be localized within certain parts of the cell. These inclusions may contain the causative chemical agent; lead, gold, iron, bismuth, uranium, beryllium, mercury, copper and arsenic are a few of the metals that can be deposited intracellularly (Sorenson and Smith, 1981). Additionally, EM methods can be used to investigate subcellular mechanisms of pollutant action. The major disadvantage of EM methods is that they are very expensive (minimum $400/fish) and very slow, requiring highly skilled technical expertise.

Histochemical Methods – Histochemical methods use the microscope in conjunction with special chemical tests and stains to localize specific enzyme systems or reaction products in the cell. For instance, histochemical assays have been used on liver tissue during and after tumor formation to yield important information on the biochemistry of specific lesions (Hinton *et al.*, 1988; Luna, 1968; Sumner, 1988). Histochemical methods are reliable and can be highly specific for certain classes of organic compounds and metals. However, highly skilled technical expertise is required to carry out the methods in the laboratory.

In vitro Tests – *In vitro* tests are generally more sensitive than whole animal systems, less expensive to carry out and of shorter duration. However, in *in vitro* tests, defense mechanisms found in the intact animal are missing. *In vitro* systems offer the greatest flexibility for the testing and study of environmental contaminants. They can be designed to be relevant to the species of interest in a given area, and multiple types of measurements can be taken from a single test system (e.g., metabolic products, mitotic activity, cytotoxicity and genetic damage). However, comparative *in vitro* and *in vivo* studies are needed to correlate and relate changes that occur in each system (Landolt and Kocan, 1983).

Reproductive/Developmental Methods

Reproduction studies are designed to examine a number of parameters in the reproductive process: gross examination of the egg and numbers of eggs, embryo viability, the proportion of fertilized eggs (i.e., fertilization success), and larval development and viability (U.S. EPA, 1986). Studies that examine the egg itself incorporate microscopic methods which are time-consuming and expensive (West, 1990), but serve as a direct measure of reproductive success. Some methods deal with the egg at the molecular level and analyze the action of chemical and physical agents whose toxicity is directed toward genetic (DNA) components of the egg.

Different cytogenotoxic tests can be used to measure a diverse array of effects including gene mutation, chromosome damage (sister chromatid exchange), primary DNA damage, or oncogenesis (i.e., tumor formation and development) (Brusick, 1980; Landolt and Kocan, 1983; Klingerman, 1982; Shugart, 1990). Another area that is currently being investigated as a suitable, sublethal assay for toxicity of certain wastes in the marine environment is the fin regeneration test (Weis *et al.*, 1990). The information collected using these methods can be used to assess the effects of pollutants on the reproductive capacity of animals. An understanding of these effects can be useful in evaluating observed population and community level changes relative to the occurrence of specific pollutants.

Gonadotropic and steroidogenic hormones regulate the reproductive capacity of an organism. The level of these hormones have been used to assess how pollutants affect the reproductive capacity of fish (Veranasi, 1990). The analysis is a sensitive indicator of exposure affecting major biological processes that impact the whole population. However, relatively detailed information about the normal reproductive cycle of the animals is necessary to apply these methods in the field (Veranasi, 1990).

Biochemical Methods

Biochemical methods have been used in field studies to measure various indicators of environmental contamination. These methods are inherently sensitive and may provide basic information about early changes in response to environmental contamination at the cellular level. The development of a suite of indicators having both specific and nonspecific responses can provide information on the type of stressors, mechanisms of action, the extent of physiological dysfunction, and potential long-term population consequences (Thomas, 1990).

Fish can respond to generalized stress, contaminants being one type of stress, through induction (increased synthesis) of stress proteins (Pickering, 1981; Sanders, 1990). Stress proteins are currently being investigated for use as generalized biochemical indicators of stress in fish, chemical-class pollutant indicators, and mode of action indicators. The methods for detecting stress proteins involve radioisotopic and immunologic methods that measure the amount of stress protein present after a stress (i.e., exposure to a pollutant) occurs. At present, cDNA probes are being used experimentally to measure the correlation between stress and induction of stress proteins. These methods potentially afford a high degree of sensitivity.

It has been suggested that induction of the fish hepatic microsomal mono-oxygenase (MO) enzyme could serve as a sensitive biological indicator for certain classes of chemicals in water (Payne *et al.*, 1987; Kleinow *et al.*, 1987; Lech *et al.*, 1982; Jimenez *et al.*, 1990; Haux and Förlin, 1988). Metallothioneins (MT) have been under consideration for use as a monitoring tool for trace environmental metal pollution due to their induction as a result of exposure to certain metals (Engel and Roesijadi, 1987; Garvey, 1990; Haux and Förlin, 1988). However, additional scientific research is required to understand the basic biology of fish before the exact significance of field studies using these techniques can be ascertained.

A concern when measuring biochemical variables in fish to detect environmental pollutants is that their exact biological significance is rarely understood. In addition, for most of the biochemical variables studied, the normal range for a particular fish population and the factors influencing these variables are often unknown (Neff, 1985). Even with these limitations, biochemical methods hold considerable promise as sensitive early indices of exposure to environmental stressors (Thomas, 1990). However, additional research is needed so that simplified, more cost effective field methodologies can be developed.

Immunological Methods

Immunological biomarkers are simple, sensitive, reproducible, and workable in the field (Weeks *et al.*, 1990; D. Anderson, 1990a; R.S. Anderson, 1990). These indicators provide supportive evidence for linkage between a stressor (toxicant, etc.) and disease outbreaks in fish and shellfish. The immune response can be used to monitor a specific antigen or microorganism responsible for pathological conditions in fish. Biologists can perform quick and sensitive assays in the field or in their own diagnostic laboratories because many immune assays are becoming available in kits (Rowley, 1990; Matthews *et al.*, 1990). Many immunological assays do not require sacrifice of the animal. Blood samples can be taken periodically to follow the kinetics of the effects of stress in a single animal; however, the effects of handling stress on aquatic species must also be considered in this case. The immune response is physiologically similar among most vertebrates and similar equipment and materials can be used to test all species of fish as well as shellfish (see Anderson, 1987). There is a rapidly growing literature on immunotoxicology from veterinary and aquatic animal sciences.

The selection of immune system indicators for the study of stress effects depends on many factors, including specific study objectives, available equipment, training, personnel, and length and number of assays. The most sophisticated and sensitive assays are costly and require highly trained personnel, whereas simple assays can be performed by field biologists with only basic laboratory supplies.

A major limitation of immune indicators is that the response is sometimes too broad to provide conclusive evidence that the observed reaction is actually due to a specific complex to be considered. Cross-reactions and heightened responses to nonspecific factors may prevent the interpretation of assays with absolute certainty. The immune response in fish or shellfish will be distinctive for a specific antigen or disease-causing agent. This will frequently make it difficult to know which immune indicator is most affected and which immunological assay to apply. Expensive materials and laboratory equipment are needed for sophisticated immunological assays. Confirmatory assays are advisable to be sure that a particular stressor is the only cause of a particular effect. Many animals should be sampled because of natural variability among individuals.

Physiological Methods

Hematological methods have been used by biologists for many years to assess the general health of fish in hatcheries and research laboratories. The procedures are well-standardized (Blaxhall and Daisley, 1973; Wedemeyer and Yasutake, 1977) and are easy to carry out, even in the field. Hematological measures may be affected by the

stress of capture, but are far less influenced than some other measurements, such as blood glucose (Larson *et al.*, 1985). Hematologic methods have been successfully implemented in the field and have been rated as the best physiologic method for evaluation of pollutants (U.S. EPA, 1986). For the measurement of hematological factors in fish, techniques similar to those used in human and veterinary clinical laboratories are generally used with minor modifications (Heath, 1987; Bouck 1984). Blaxhall and Daisley (1973), Wedemeyer and Yasutake (1977), and Ellis (1977) provide practical guides to the adaptation of these methods for use on fish blood.

The hematocrit/erythrocyte determination may not be as sensitive to pollution as is the leucocyte count, at least as far as its response to metals is concerned (Larson *et al.*, 1985). It remains to be determined how sensitive the procedure is to subtle environmental changes. In general, chemical and physical stressors cause a decrease in the leucocrit, whereas infections produce the opposite response. However, it is also possible to get elevations in granulocytes concomitant with a decrease in lymphocytes, thereby yielding an unchanged leucocrit (Peters *et al.*, 1980). Thus, the method has its limitations for the detection of chronic stress (Wedemeyer *et al.*, 1983).

There are several obstacles, such as capture stress, that limit the potential usefulness of physiological tests in field work. Capture stress precludes the use of sensitive, early indicators of environmental stress. The use of physiological responses of wild fish to assess environmental quality is difficult because responses due to toxicants often cannot be distinguished from those induced during handling of the wild fish (Bouck, 1984).

B9.4 QA/QC Considerations

Pathobiological methods have a wide range of sensitivities and response times (i.e., when a response can be detected). For instance, certain immunologic, electron microscopic, and biochemical methods can detect early changes in cells and are very sensitive. Gross anatomic pathology methods, on the other hand, can only detect cellular changes after lesions can be seen and, therefore, are less sensitive.

General considerations for QA/QC procedures have been covered earlier in this document (Section B.1). With regard to pathobiological methods, it should be noted that careful and consistent handling of aquatic specimens is required to minimize trauma and confounding effects, such as exposure to air. Organisms should be held in the laboratory under conditions as near to those found at the site of collection as possible and for as short a time as practicable before performing assays. Fish and shellfish collected for histopathological examination must be properly fixed (e.g., immersed in a formaldehyde or glutaraldehyde solution) to stop metabolic activity.

This may require opening or sectioning the organisms to allow the fixative to rapidly penetrate all tissues and preserve the cellular structure in its existing condition. The organisms must still be active or moribund, but not dead before being fixed. Failure to follow proper fixation procedures will interfere with the interpretation of lesions in anatomic pathology studies.

As with other monitoring methods, samples must be accurately labeled at the time of collection and routine QA/QC procedures should be instituted, including tracking of samples, careful recording of methods used, using fresh solutions, and treating both control and exposed samples equally. Whenever possible, organisms for each group to be tested should be of the same species, age, and sex. Sections of tissues and organs, or for small organisms, the whole animal, should also be prepared as uniformly as possible with respect to homogeneity and orientation so that microscopic observations can be made on the same organs and areas for each specimen. Subsamples of sections should be examined (blind) by another pathologist to confirm the diagnoses. See U.S. EPA (1987a) for additional information on QA/QC procedures for histopathological examinations.

B9.5 Statistical Design Considerations

Statistical strategies may mitigate the high costs of pathobiological monitoring methods. As discussed earlier, power analyses considering the strategy of compositing samples can often lead to a cost-effective monitoring design strategy. Power-cost analyses are necessary in selecting the appropriate sample/replicate number, sample location, and sampling frequency. If the primary objective of a monitoring program is to determine pathobiological differences among sampling locations, composite sampling may be an appropriate strategy. However, a limitation of composite sampling is the inability to directly estimate the range and variance of the population of individual samples. Similarly, the use of space- or time-bulking strategies will severely limit a monitoring program's ability to assess spatial and temporal heterogeneity of the samples. See Statistical Design Considerations: Composite Sampling, Statistical Power, and Power-Cost Analysis (Section B.3).

Given that the monitoring program must accommodate a fixed level of sampling cost, the best strategy for pathobiological monitoring is to collect more replicates at fewer locations. For histopathology, from 25 to 100 animals per species may need to be surveyed to detect low incidences of disease. The selection of appropriate statistical analyses must be made with these limitations in mind, as well as the specific types of statistical analyses that can be performed on different types of data. For example, quantitative information from measurements of enzyme levels or computerized image analysis/morphometric programs may be effectively analyzed by parametric statistics

(if the data fit the conditions for normal distributions, limitations of assumptions, etc.), but histopathological observations may need to be rated on presence/absence or categorized qualitative scales that will require non-parametric techniques. U.S. EPA (1987a) provides additional information on the types of sampling designs and statistical tests that may be appropriate for these data.

B9.6 Use of Data

Data Interpretation

Data interpretation for pathobiological methods may be limited because there are relatively few trained personnel, facilities and equipment may be expensive, and references for techniques that are newly emerging may be scarce. However, because of the recent interest in developing biomarkers for monitoring the effects of natural and anthropogenic environmental stresses on aquatic organisms, many research programs are underway at Federal, State, and academic facilities to develop, standardize, and validate the most promising biomarkers for sentinel species that will establish cause and effect links for pollutant exposure. Basic laboratory research and experimental studies must be conducted in conjunction with field work to elucidate the relationships between contaminant levels, structural, biochemical, or functional pathologies, and population health (Johnson and Bergman, 1984; McCarthy, 1990). A critical concern is the coordination of efforts and creation of a multidisciplinary approach.

It is important to establish baseline data on selected species as well as to demonstrate the alterations in the health of those species due to contaminant exposures. Methods and techniques, terminology, and interpretation of lesions and effects must be standardized. In addition to a rapidly expanding body of literature on baseline measures of health and histological atlases for several species of fish and shellfish, and courses, workshops, meetings, and special symposia (e.g., Responses of Marine Organisms to Pollutants/Woods Hole, MA and Plymouth, England; Annual Aquatic Toxicology Workshop, Canada), several professional societies (e.g., Society for Invertebrate Pathology, American Fisheries Society/Fish Health Section) are facilitating training in techniques and communication among investigators and laboratories. Diseases of fish and shellfish have been reviewed by several authors (i.e., Sparks, 1985; Ferguson, 1989; Roberts, 1989; Sindermann, 1990). For histopathological studies of tumors in fish, standardization of nomenclature will be available in Dawe *et al.* (in press).

As for any monitoring program, long-term studies of health and disease in aquatic organisms will aid in identifying and interpreting observed pathobiological effects. Furthermore, it will be important to archive data so that it will be available for future

comparisons. The Registry of Tumors in Lower Animals at the National Museum of Natural History, Smithsonian Institution, Washington, D.C. houses over 3500 cases of neoplastic and nonneoplastic lesions representing a wide variety of host aquatic species, pathogens, and environmental stresses from field and laboratory studies conducted around the world. The Registry also contains the Registry of Marine Pathology, originally developed by the National Marine Fisheries Service (NMFS), and the collection of crustacean histopathological material researched by Dr. Phyllis T. Johnson, NMFS. Although primarily serving as a clearinghouse for information on neoplastic diseases and research, the Registry is also able to direct inquiries for information on nonneoplastic diseases, and the materials archived there are available for study by qualified investigators. Data collected from the long-term monitoring efforts of NOAA's National Status and Trends Program (NS&T) and the EPA's Environmental Monitoring and Assessment Program (EMAP) will also provide useful information for the interpretation of the various pathobiological methods that are being employed in these studies.

Data Integration

Measuring or evaluating the effects of stressors on fish and shellfish by pathobiological indicators is difficult primarily because of the large number of variables that can influence biological response. Variables including water temperature, nutritional status, species, sex, reproductive and developmental stages, and physiological functions can render tests difficult to compare and evaluate.

Anderson (1990a) outlined a plan including four levels of study in a tiered approach to investigate the effects of stress on the immune system and to quantify the possible contribution of these environmental and physiological variables on the stress response. The plan, generally speaking, appears to be applicable to all pathobiological methods and may be adapted to them using the following four levels: (1) observations of fish and shellfish populations in the field; (2) studies of caged fish or shellfish in the field; (3) *in vivo* exposures in the laboratory; and (4) *in vitro* assays in the laboratory.

The first level of study includes the collection of information from field observations on relative abundance, reduction in sport-fish catches, or decline in commercial harvests. Reduced yields reported by anglers in previously productive fishing grounds may be correlated with environmental stressors or pollutants occurring at these sites. If sick or moribund fish or shellfish are called to the attention of local biologists by anglers, gross morphological descriptions might be made and isolation of the disease agent attempted.

The second level of investigation involves the use of caged fish or shellfish placed in the field to test for the presence or action of environmental stressors. Groups of fish or shellfish can be placed in pens or cages at suspected sites and their responses (signs of disease, acute mortality) can be compared with those of control fish or shellfish caged in unstressful areas. Organisms from the cages can be sampled at various intervals and the intensity of various immune responses quantified as a function of the time the fish or shellfish were exposed. Levels of immune responses can be compared by injecting specific disease agents into both control and test organisms, and recording disease and death rates.

The third level of assay is *in vivo* laboratory tests where immune response can be evaluated in experiments with calibrated dilutions of specific contaminants and kinetic measurements of the immune response at each dilution. Challenges with disease agents can be more easily controlled in the laboratory to provide information on how the stressor makes the fish or shellfish more susceptible to specific pathogens.

A fourth level of investigation is the recently developed method for testing the effects of chemicals, drugs, and other stressors *in vitro*. Spleens and other immunopoietic organs can be removed from fish and placed in tissue culture media and their reactions to pollutants (Anderson *et al.*, 1986) tested using the passive hemolytic plaque assay. Use of this method to measure the effects of stressors allows maximal control of the levels of pollutants. Because the immune response is monitored under defined laboratory and environmental conditions, important information is obtained about how specific stressors affect the immune response. *In vitro* methods require fewer fish than *in vivo* methods because tissue and organ samples can be divided into many sections, which also reduces the variability of responses.

McCarthy (1990) presented a research strategy to validate biomarkers and provide the scientific understanding necessary to interpret biomarker responses. An evolving monitoring program was proposed that focused broadly on evaluation of contamination in an array of ecosystem types. The challenges and obstacles to be addressed in such a program include the following:

- The quantitative and qualitative relationships between chemical exposure, biomarker response, and adverse effects must be established

- Responses due to chemical exposure must be distinguishable from natural sources of variability (e.g., ecological and physiological variables, species-specific differences, and individual variability) if biomarkers are to be useful in evaluating contamination

- The validity of extrapolating between biomarker responses measured in individual organisms and some higher-level effect at a population or community level must be established

- The use of exposure biomarkers in animal surrogates to evaluate the potential for human exposure should be explored

The plan is an ambitious multi-year research and development program involving the formulation of a long-term, interagency, interdisciplinary activity such as the Environmental Monitoring and Assessment Program (EMAP) by EPA in cooperation with other federal agencies.

Rationale

- Pathobiological methods provide information concerning biological organ systems that, through an evaluation of organ structure, activity, and function, can be used to determine adverse effects of pollutants in the environment.

- Pathobiological methods should be used in concert so that cause and effect type relationships can be evaluated.

B9.7 Summary and Recommendations

Monitoring Design Considerations

- Sampling stations should be located along a contamination gradient (i.e., from highly contaminated to uncontaminated). This type of sampling strategy will allow dose-response relationships to be evaluated.

- Fish pathobiological monitoring should be conducted only if the target species could reasonably be expected to have spent a considerable amount of time within the area of contamination.

- Large sample sizes will frequently be required for fish pathobiological monitoring due to natural variability among individuals and taxa.

Existing Analytical Methods

Anatomic Pathology Methods–

- Tissues are examined with the naked eye or the aid of a microscope
- Light microscopic (LM) methods
  - detect changes at the cellular level
  - organ and time-series specific
- Electron microscopic (EM) methods
  - detect subcellular changes
  - specific as to area of cell or site of pollutant action
- Histochemical methods
  - detect enzyme systems or reaction products in the cell
  - specific to chemical class

Reproduction/Development Methods–

- Reproductive capacity is reflected in population recruitment and abundance
- Methods that examine the egg itself provide a direct measure of reproductive effects from pollutants
- Cytogenetic (DNA) tests
  - *in vitro* tests offer greatest flexibility in terms of sensitivity, expense, and time required, and are frequently conducted for convenience and availability
  - sister-chromatid exchange (SCE) assays are DNA damage tests used as a screening tool, and are dose-responsive and sensitive to low concentrations of pollutants
  - the aneuploidy technique is simple and easy to use, but is inaccurate for sublethal effects
  - chromosomal alteration analysis

Biochemical Methods–

- Several methods are specific for a certain compound class

- Use a suite of indicators for environmental monitoring

- Microsomal mono-oxygenase (MO) assays could serve as sensitive biological indicators for certain chemical classes

- Metallothionein (MT) assays are nonspecific for metal exposure, but can provide information on the likelihood of a particular metal pool producing a pathological effect

- Stress proteins are not pollutant-specific, but could result from generalized stress

Immunologic Methods–

- Immunologic assays can provide information on pollutant-induced stress effects; however, they may require confirmatory assays

- Many animals should be sampled because of natural variability among animals

Physiologic Methods–

- Serious "interferences" can be caused by stress induced during collection and may limit the potential usefulness of physiological tests because effects of toxicants cannot be distinguished from those induced during handling of the wild organisms

- Hematologic methods for fish, such as measurements of hematocrit (packed cell volume), hemoglobin concentration, erythrocyte count and leucocyte count (or volume) can be successfully used, although the fish are not immune to stress of capture, hematologic methods are influenced far less than some other measurements such as blood glucose

- The leucocrit can be a more sensitive measure of metal pollution than the hematocrit

QA/QC Considerations

- Pathobiological methods have a wide range of sensitivities

- Organisms must be carefully handled and properly prepared for each method

- The main areas of concern with regard to analytical QA/QC are precision, accuracy, representativeness, completeness, and comparability

Statistical Design Considerations

- Composite tissue sampling consists of mixing tissue samples from two or more individuals collected at a particular location and time

- Space- (combining composites from several locations) and/or time- (combining several composites over time from one location) bulking strategies should be used judiciously since information concerning spatial and temporal heterogeneity may be lost

- Power analyses have shown that for a fixed level of sampling effort, a monitoring program's power is generally increased by collecting more replicates at fewer locations

- The appropriate statistical tests must be performed and may vary depending on the type of data generated

Use of Data

- Data use and interpretation may be limited by relatively few trained personnel and expensive equipment, but many research and training programs are now underway at Federal, State, and academic facilities to improve biomarker methods in sentinel species

- Basic laboratory research must be conducted and biological methods must be tested in the field

- Information communication
  - need to coordinate efforts, communicate and create a multidisciplinary approach to relate lab and field studies and establish cause and effect type relationships

Data integration

long-range research strategies should be followed to validate biomarkers and provide scientific understanding necessary to interpret biomarker responses

B9.8 Literature Cited and References

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B10.0 Bioaccumulation

Bioaccumulation is the overall process of biological uptake and retention of chemical contaminants obtained from foods, water, sediments, or any combination of exposure pathways – a list of terms may be found in Table B10-1. It is a consequence of an organism's physiological limitations to transform and excrete the invading chemical substances.

TABLE B10-1. LIST OF TERMS

Bioavailability	=	the fraction of the total contaminant in the surrounding medium which is correlated with a quantitative biological response, such as bioaccumulation (Landrum and Robbins, 1990; DeWitt, 1991)
Bioaccumulation	=	uptake and retention of substances by an organism from its surrounding medium and from food (U.S. EPA, 1990a)
Bioconcentration	=	uptake of substances by an organism from the surrounding medium through gill membranes or other external body surfaces (U.S. EPA, 1990a)
Biomagnification	=	the process by which the concentration of a compound increases in different organisms, occupying successive trophic levels (U.S. EPA, 1990a)

B10.1 Rationale

The presence of toxics in the waters and sediments of estuaries can have adverse ecological and human health effects. Potential consequences of bioaccumulation of chemical contaminants in estuarine organisms include, but are not limited to:

- significant mortality to susceptible estuarine organisms
- lethal or sublethal chronic toxic responses at later stages of the life cycle or under conditions of stress for susceptible estuarine organisms
- the transfer of increasingly greater concentrations of toxic pollutants to organisms at higher trophic levels – including humans

It is difficult to calculate the uptake and bioaccumulation of contaminants from measured values in the water and sediments. Contaminants often occur in the water

column at very low concentrations which are difficult to detect, yet can be accumulated in detectable amounts in fish and shellfish tissue. In those cases where toxics can be measured in the water and sediments, there is a large degree of uncertainty associated with even the most sophisticated models for predicting uptake and bioaccumulation. Direct monitoring of the concentration of contaminants of concern in the tissue of selected estuarine organisms can provide a spatial and temporal record of contaminant concentrations and bioaccumulation in the estuary.

Monitoring of bioaccumulation in the estuary will provide the data which links exposure and effects, and thus can generate important insights into ecological effects, human health risks, and routes and extent of pollutant exposure. This information can be used to both evaluate the effectiveness of the CCMP in mitigating the potential effects due to bioaccumulation and to provide an early warning system for the potential risks associated with the bioaccumulation of xenobiotics.

B10.2 Monitoring Design Considerations

Bioaccumulation monitoring studies have varied substantially, depending upon study-specific objectives. Generally, bioaccumulation monitoring programs whose objective was to assess human health effects were significantly different in their selection of target species and tissues when compared to those which were intended to assess impacts to estuarine communities. Human health risk assessments usually examine the muscle tissue of commercially important fish and shellfish, whereas environmental impact assessments often survey organisms at lower trophic levels (e.g., infaunal macroinvertebrates). Unfortunately, formulations which would allow comparisons of bioaccumulation data between different species and/or different tissues types have not yet been developed. Thus, it is essential that monitoring design elements be standardized to allow for comparisons among estuarine studies.

A common deficiency in many programs, is the inability to collect sufficient tissue biomass of appropriate species across sampling locations throughout the study. The selection of appropriate species and tissues must account for natural fluctuations in populations as well as changes due to anthropogenic perturbations. Indigenous species initially present may not be available later, limiting temporal and spatial comparisons.

Selection of Target Species

A key component of any bioaccumulation monitoring program is the selection of target species. Concentrations of chemical residues in tissues of target species serve as indicators of contamination throughout the biological system. The fundamental

criterion is the ability to use the selected species to make comparisons between sampling locations and sampling periods.

Target Species Characteristics – It is recommended that the target species possess the following characteristics (U.S. EPA, 1985a):

- high bioaccumulation potential for selected contaminants of concern
- metabolic regulation of selected contaminants should be weak or absent in order to assess a "worst case scenario"
- abundant, temporally and spatially, to allow for adequate sampling
- large enough to provide adequate amounts of tissue for analysis
- sessile or sedentary in nature to assure bioaccumulation is representative of the study area
- easily collected
- existing database of exposures and sensitivity

The EPA has provided a list of suggested fish and macroinvertebrate target species (Tables B10-2 and B10-3; U.S. EPA, 1985a) for different regions in the United States.

Secondary considerations, based on economic importance and status as a bioassay organism, may be applied to further winnow the list of candidate target species to a practical number of species to be analyzed.

Candidate target species should be objectively ranked based on ecological characteristics which would enhance their potential for bioaccumulation, and facilitate sampling and/or analytical procedures (U.S. EPA, 1985a). Macroinfauna have several advantages as target species, including:

- are sedentary; therefore, bioaccumulation will be representative of the study area
- represent a significant food source for higher trophic levels and therefore may indicate contaminants available in the food chain

TABLE B10-2. HIGHEST RANKING CANDIDATE FISHES FOR USE AS BIOACCUMULATION MONITORING SPECIES

State	Locality	Species	Secondary Selection Criteria: Economic Importance	Secondary Selection Criteria: Bioassay Species
MASSACHUSETTS	Swampscott	Winter flounder	Yes	Yes
		Yellowtail flounder	Yes	Yes
		Ocean pout	No	No
		Windowpane	No	No
	Lynn	Winter flounder	Yes	Yes
		Yellowtail flounder	Yes	No
		Ocean pout	No	No
	South Essex	Winter flounder	Yes	Yes
		Yellowtail flounder	Yes	No
		Windowpane	No	No
		American eel	No	No
		Ocean pout	No	No
	Boston	Winter flounder	Yes	Yes
		Yellowtail flounder	Yes	No
		Ocean pout	No	No
		Windowpane	No	No
	Fall River	Winter flounder	Yes	Yes
		Windowpane	No	No
	New Bedford	Winter flounder	Yes	Yes
		Scup	Yes	No
		Summer flounder	Yes	Yes
RHODE ISLAND	Newport	Winter flounder	Yes	Yes
		Scup	Yes	No
		Weakfish	Yes	No
NEW YORK	Upper East River	Winter flounder	Yes	Yes
		Windowpane	No	No
		Weakfish	Yes	No
	Lower East River	Spot	Yes	Yes
		Scup	Yes	No
		American eel	No	No
	Lower Hudson River	Hogchoker	No	No
NEW JERSEY	Cape May	Spot	Yes	Yes
		Red hake	No	No
		Windowpane	No	No
		Summer flounder	Yes	Yes
VIRGINIA	Portsmouth	Spot	Yes	Yes
		Summer flounder	Yes	Yes
		Atlantic croaker	Yes	No
		Hogchoker	No	No
	Virginia Beach	Spot	Yes	Yes
		Red hake	No	No
		Summer flounder	Yes	Yes

TABLE B10-2.
(continued)

State	Locality	Species	Secondary Selection Criteria: Economic Importance	Secondary Selection Criteria: Bioassay Species
CALIFORNIA (NORTHERN)	San Francisco	English sole	Yes	Yes
		Pacific sanddab	Yes	No
		Big skate	No	No
	Oakland	English sole	Yes	Yes
		Starry flounder	Yes	No
		Pacific staghorn sculpin	No	No
	Monterey	English sole	Yes	Yes
		Curlfin sole	Yes	No
	Santa Cruz	English sole	Yes	Yes
	Watsonville	English sole	Yes	Yes
		Curlfin sole	Yes	No
CALIFORNIA (SOUTHERN)	Goleta	Dover sole	Yes	No
		Pacific sanddab	Yes	No
		Longspine combfish	No	No
		Spotted cusk-eel	No	No
	Santa Barbara	English sole	Yes	Yes
		Pacific sanddab	Yes	No
	L.A. County	Dover sole	Yes	No
		Curlfin sole	Yes	No
		English sole	Yes	Yes
	Orange County	Dover sole	Yes	Yes
		Pacific sanddab	Yes	Yes
		English sole	Yes	Yes
	Hyperion	Dover sole	Yes	No
	Oceanside	Longspine combfish	No	No
		Big skate	No	No
	Escondido	California skate	No	No
	San Elijo	Dover sole	Yes	No
		Blackbelly eelpout	No	No
		Pacific sanddab	Yes	No
		English sole	Yes	Yes
	San Diego	Dover sole	Yes	No
		English sole	Yes	Yes
		Pacific sanddab	Yes	No
		Longspine combfish	No	No
WASHINGTON	Central Puget Sound	English sole	Yes	Yes
		Dover sole	Yes	No
		Rock sole	Yes	No
		Spotted ratfish	No	No
		Rex sole	Yes	No
		C-O sole	Yes	No

TABLE B10-3.
RECOMMENDED LARGE MACROINVERTEBRATE SPECIES FOR BIOACCUMULATION MONITORING

Region	Recommended Species[a]
Massachusetts to Virginia	American lobster (*Homarus americanus*)
	Eastern rock crab (*Cancer irroratus*)
	Hard clam (*Mercenaria mercenaris*)
	Soft-shell clam (*Mya arenaris*)
	Ocean quahog (*Artica islandica*)
	Surf clam (*Spisula solidissima*)
	Edible mussel (*Mytilus edulis*)
Alaska to California	Spiny lobster (*Panulirus interruptus*)
	Dungeness crab (*Cancer magister*)
	Rock crab (*Cancer antennarius*)
	Yellow crab (*Cancer anthonyi*)
	Red crab (*Cancer productus*)
	California mussel (*Mytilus californianus*)
Florida, Virgin Islands, and Puerto Rico	Spiny lobster (*Panulirus argus*)
Hawaii	Spiny lobster (*Panulirus penicillatus*)

[a] Additional species that may occur at specific discharge sites and are considered acceptable bioaccumulation monitoring species include the American oyster (*Crassostrea virginica*) and the Pacific oyster (*Crassostrea gigas*).

However, macroinvertebrates may not provide sufficient tissue biomass for analyses. Adult fish of the species listed in Table B10.2 provide sufficient tissue biomass for analyses and are direct measures of contaminants available to human populations. However, fish are usually motile and bioaccumulation measured may not be representative of the study area.

It is recommended that bioaccumulation studies be conducted for a number of species.

Species have different bioaccumulation potentials for various contaminants; monitoring multiple species will ensure that bioaccumulation of a number of contaminants are sufficiently evaluated. For example, oysters and other bivalves are ideal for monitoring bioaccumulation of PAH's due to their limited ability to metabolically transform them. Monitoring of species along a food chain might provide information concerning the transfer of contaminants to higher trophic levels. Species with extensive historical bioaccumulation data are also preferred. Where possible, species being collected for other aspects of the monitoring program (e.g., fisheries data, pathobiology, pathogens) should be included in the bioaccumulation analyses, to enhance the coordinated evaluation of data.

Caged Indicator *vs.* Indigenous Species – The California Mussel Watch Program, the U.S. Mussel Watch Program, and the National Oceanic and Atmospheric Administration (NOAA) Status and Trends Program have employed the use of both resident and caged transplant mussels to monitor bioaccumulation of toxic chemicals over space and time (Goldberg *et al.*, 1978; Boehm, 1984; Ladd *et al.*, 1984). Caged indicator species offer several advantages over indigenous species:

- the biology and ecology of these indicator species are usually well described
- the descriptions of culture and/or maintenance of the organisms under laboratory conditions are often available
- the method provides control of initial temporal and spatial variation of individuals and/or biomass
- the method allows the use of a specific age, size and/or genetic stocks
- the method assures on-site bioaccumulation

By controlling for initial conditions, the rate of change of tissue contamination may be calculated. In addition, the Long Island Sound Estuary Program has shown that chemical residue analyses on caged indicator species appears to be a promising approach for identifying sources of pollution (U.S. EPA, 1982). However, transfer through the water is the only exposure pathway assessed; adequate methods for caging sediment ingesting organisms are not yet available. Because bioaccumulation of sediment-sorbed contaminants is probably mediated by transfer through interstitial waters (Knezovich and Harrison, 1987) and results obtained often do not relate to species found on site, caged organisms may not provide an accurate estimate of the bioavailability of certain contaminants occurring at the site.

The advantages of indigenous species are:

- results obtained will relate directly to those species which may be impacted and provide a direct measure of potential risks to human health
- no cost of transplanting organisms
- no possibility of loss of cage/buoy system
- no possibility of introduction of a "nuisance" species

However, common indigenous species may not meet the criteria for use as bioaccumulation target species, negating the advantage of using a native species. The most common problem is collecting sufficient tissue biomass of appropriate indigenous species across sampling locations throughout the study.

Selection of Tissues

The type and location of tissue analyzed will depend upon the objectives of individual monitoring programs. It is essential that all analyses be conducted with the same tissue type from the same species in order that scientifically and statistically valid comparisons may be made.

Target Tissues – In fish, liver tissue is closely associated with regulation and storage of many toxic substances (Fowler, 1982). Its high fatty tissue content tends to accumulate hydrophobic contaminants. Thus, contaminant levels in the liver can be used to estimate the range of contaminants being assimilated. For macroinvertebrates, hepatopancreas or digestive gland tissue performs functions analogous to fish liver tissue.

Contaminants in edible muscle tissue represent those contaminants that are retained in a form that allows transfer to humans. Sampling of muscle tissue is appropriate for human exposure assessments and quantitative health risk determinations. Within a fillet, contaminant concentrations may vary, therefore it is recommended that a consistent location within the muscle tissue be analyzed (U.S. EPA, 1989).

Whole body analyses ought to be conducted when predators consume the whole body of the target organism. If organisms are not cleansed of materials contained in the digestive tract, contaminants in the gut contents will be included in the analyses. To provide the most accurate estimate of the total amount of contaminants available to most macroinvertebrate predators, this type of depuration may not be required.

Sediment Total Organic Carbon and Acid Volatile Sulfides, and Tissue Lipid Normalization – Toxic sediment concentrations of hydrophobic contaminants have been found to be related to the total organic carbon content (TOC) of the sediment (Karickhoff *et al.*, 1979). The bioavailability of some metal contaminants has been found to be related to the acid volatile sulfide (AVS) concentrations of the sediment (DiToro *et al.*, in press; see Section B4.2). TOC and AVS normalizations have been conducted to estimate the concentrations of sediment contaminants which are bioavailable over different sampling locations. Sediment TOC and AVS normalizations are recommended to account for the variability in bioavailable sediment contaminant concentrations between locations.

Lipids appear to be a storage site of organochlorines, hydrocarbons, and other hydrophobic contaminants in a variety of organisms (de Boer, 1988). In fact, just as TOC and AVS are considered by many to be important parameters in defining organic and metal concentrations in sediments, lipid content is considered the salient parameter in defining hydrophobic residue concentrations found in tissues (Phillips and Segar, 1986). Likewise, tissue lipid normalizations have been suggested to account for the variability in tissue contaminant concentrations between individuals.

Normalization of sediment contaminant concentrations to TOC, AVS, and tissue contaminant concentrations to the tissue lipid concentrations have been made to permit comparisons of toxic chemical residues in tissues between studies, locations, individuals, and tissue types. These sediment and tissue normalizations assume the following:

- contaminants partition predominantly to sediment TOC, sediment AVS, and organismal lipids
- contaminants partition reversibly between the sediment particles and organism
- rapid steady-state kinetics of contaminants
- sediment is the only source for the bioaccumulation

Lipid normalization has been performed in order that individuals with differing body fat levels may be compared. Again, it is essential that all analyses be conducted with the same tissue type from the same species in order to ensure that scientifically and statistically valid comparisons may be made. TOC/lipid normalized accumulation factors (AF) have also been used to predict tissue residue concentrations (Ferraro *et al.*, 1990; Lake *et al.*, 1987).

Three-fold differences in lipid concentration may result as a consequence of various lipid analysis techniques. Development of protocol standardization or intercalibration between lipid techniques are required before chemical residues in tissues can be compared among studies. Microtechniques have been developed for analyses of lipids from single individuals (Gardner *et al.*, 1986).

Finally, the decision to normalize for TOC, AVS, and percent lipid will depend upon monitoring program objectives. For example, if the monitoring objective is to identify "hot spots" – i.e., those areas where high body burdens present a risk to human and ecological health – normalization may not be appropriate. Whereas, if the objective is to identify temporal trends in bioaccumulation rates, normalization may be justified.

Selection of Sampling Period

The timing of sampling should be based on biological cycles which influence an organism's susceptibility to bioaccumulation. The frequency of sampling should be related to the expected rate of change in tissue concentrations of contaminants. A consistent sampling period is recommended in order that spatial and temporal comparisons may be conducted.

For crustaceans, just after molting, before hardening of the integument occurs restricting its permeability, there is a significant increase in potential for bioaccumulation of toxic contaminants.

For many aquatic vertebrate and invertebrate organisms, the reproductive cycle exerts a major influence on tissue concentrations of many contaminants – especially hydrophobic compounds (Phillips, 1980). For many species, lipophilic (hydrophobic) contaminants are transferred from the muscle and liver to the eggs as lipids are mobilized and transported to the egg during oogenesis (Spies *et al.*, 1988; Gardner *et al.*, 1985). There is evidence that lipophilic contaminants have deleterious effects on the developing egg and/or the larvae (Spies *et al.*, 1988; Hansen *et al.*, 1985; Niimi, 1983). Transfer of contaminants from adult to egg, and its potential impacts to future fisheries recruitment and fisheries production are under investigation.

It is recommended that target species be sampled when tissue contaminant concentrations are expected to be at their highest level in order to evaluate worst-case scenarios. For those organisms where the body is consumed whole, contaminant levels are usually at their highest at or just before spawning. For organisms where the muscle tissue is consumed, contaminants in muscle tissue usually reach a peak well before spawning.

B10.3 Existing Analytical Methods

Questions to be considered during the choice of an appropriate analytical method include the parameters of interest, desired detection limits, sample size requirements or restrictions, methods of preservation, technical and practical holding times, and matrix interferences. Several U.S. EPA documents (1986a, 1987a, 1990b) discuss the common analytical problems encountered during monitoring analyses of tissue samples.

Chemical Residue Analyses

Several factors determine achievable detection limits for a specific contaminant, regardless of analytical procedure – a list of analytical procedures and EPA method numbers is given in Table B10-4. These factors include:

- size of the tissue available for analysis – in general, the ability to detect low contaminant levels is improved with greater amounts of tissue available for analysis; a minimum of 30 g (wet weight) is usually considered adequate (U.S. EPA, 1987a)
- presence of interfering substances
- range of pollutants to be analyzed – an optimal method for a specific pollutant may be developed without regard to potential effects on other parameters
- level of confirmation – qualitative (e.g., presence or absence) or quantitative (e.g., residue concentrations) analyses
- level of pollutant found in the field and in analytical blanks

Selection of appropriate methods ought to be based on a trade-off between full-scan analyses, which are economical but cannot provide optimal sensitivity for some compounds, and alternate methods that are more sensitive for specific compounds but can result in higher analytical costs.

Metals and Metalloids – Nitric acid/perchloric acid digestions should be considered for analysis of tissue samples (U.S. EPA, 1987a). Perchloric acid is especially useful for the dissolution of fats.

Trace element analyses by ICP (U.S. EPA Method 6010) allows for several elements to be measured simultaneously. Detection limits of ICP for most metals are generally

comparable to those achieved by graphite furnace AAS (GFAAS); however, detection limits for several metals are significantly lower using AAS: arsenic, selenium, and mercury.

GFAAS is the recommended analytical method for detection of arsenic, selenium, lead and thallium in tissues (U.S. EPA, 1987a). Cold vapor AAS (U.S. EPA method 7470) analysis is the only recommended technique for mercury (U.S. EPA, 1986a).

GFAAS is more sensitive (i.e., lower detection limits) than flame AAS, but is more subject to matrix and spectral influences – GFAAS requires particular caution with regard to laboratory contamination. GFAAS requires more skilled laboratory technicians. Both AAS methods require that the concentration of each element be determined by a separate analysis, making the analysis of a large number of contaminant metals both labor-intensive and relatively expensive compared to ICP. ICP is therefore the preferred method of analysis for metals, except arsenic, selenium, lead, mercury and thallium.

Semi-Volatile Organic Compounds – Analysis of semi-volatile organic compounds involves a solvent extraction of the sample, cleanup of the characteristically complex extract, GC analysis, and quantification (U.S. EPA, 1987a; U.S. EPA, 1986a). There are two GC/MS options for detecting extractable organic compounds: internal standard technique and isotope dilution. The isotope dilution technique is recommended because reliable recovery corrections can be made for each analyte with a labeled analog or a chemically similar analog (U.S. EPA, 1986a). The isotope dilution method is more expensive and less widely employed than the internal standard technique, which is the current method of choice in the Contract Laboratory Program (CLP). Mass spectrometry provides positive compound identification by comparison of both retention time and spectral patterns with standard compounds.

The identification of pesticides and PCB congeners can be made by GC/ECD analysis (U.S. EPA Method 8080). GC/ECD provides greater sensitivity relative to GC/MS, however GC/ECD does not provide positive compound identification. Confirmation of pesticides and PCBs on an alternative GC/ECD or preferably by GC/MS, when sufficient concentrations occur, is recommended for reliable results (U.S. EPA, 1985b). All other organic compound groups are recommended for analysis by GC/MS.

Volatile Organic Compounds – Analysis of volatile organic compounds also involves GC analysis and quantification (U.S. EPA, 1987a; U.S. EPA, 1986a). The purge and trap GC/MS technique is employed for detecting volatile organic compounds in water.

TABLE B10-4. LIST OF EXISTING ANALYTICAL TECHNIQUES (U.S. EPA, 1986b)

METALS/METALLOIDS	
• Atomic Absorption Spectrophotometry (AAS)	US EPA Method 7000 series
- flame	
- graphite furnace (GFAAS)	
- cold vapor	US EPA Method 7470
-gaseous hydride (HYDAAS)	US EPA Methods 7060 and 7740
• Inductively Coupled Plasma Emission Spectrometry (ICP)	US EPA Method 6010
ORGANICS	
• Gas Chromatography (GC)	
-with electron capture detection (GC/ECD)	US EPA Method 8080
-with mass spectrometry (GC/MS)	US EPA Methods 8240 and 8270

A successful variation for detection of volatile organic residues in tissues involves a device that vaporizes volatile organic compounds from the tissue sample under vacuum and then condenses the volatiles in a super-cooled trap (Hiatt, 1981). The trap is then transferred to a purge and trap device where it is treated as a water sample. The isotope dilution option is recommended as it provides reliable recovery data for each analyte (U.S. EPA, 1986a). However, this technique creates additional analytical time and expense.

B10.4 QA/QC Considerations

Sample Collection

In the field, sources of contamination include sampling gear, lubricants and oils, engine exhaust, airborne dust, and ice used for cooling samples. Samples designated

for chemical analyses require all sampling equipment – e.g., siphon hoses, scoops, containers – be made of noncontaminating material and be cleaned appropriately prior to use. Efforts should be made to minimize handling and to avoid sources of contamination including potential airborne contamination (e.g., stack gases, cigarette smoke).

To avoid contamination from ice, whole samples should be placed in watertight plastic bags, and immediately cooled in a covered ice chest. Only if the samples will not be analyzed for metals, they may be wrapped in acid-rinsed aluminum foil with the shiny side out before being placed in plastic bags. Furthermore, samples and sampling containers should not be touched with ungloved fingers.

Sample Handling and Storage

For analyses of metals, samples should be frozen and kept at -20°C (Table B10-5). Although specific tissue sampling holding times have not been recommended by the EPA, a maximum of 6 months (8 days for mercury; ASTM, 1991) would be consistent

TABLE B10-5.
SAMPLE PRESERVATION AND STORAGE PARAMETERS

	Sample		Storage	
Analyte	Container[a]	Size	Preservative	Lifetime
All metals (except Hg)	P,G	5 g[b,f]	Freeze[d]	6 mo[c]
Hg	P,G	0.2 g[b,f]	Freeze[d]	8 days[c]

[a] P = linear polyethylene, G = borosilicate glass, TFE = tetrafluoroethylene.

[b] Wet weight.

[c] Suggested. No EPA criteria exist. Hg holding time 8 days.

[d] Post-dissection.

[e] If aliquot for Hg taken from this 1 liter sample, cannot use linear polyethylene.

[f] Weight is a minimum for one sample. Studies using specific organs may require more.

with that for water samples. For analyses of volatile compounds, samples should be stored in the dark at 4°C (Table B10-6; U.S. EPA, 1987a). Analyses of volatile compounds should be performed within 14 days of collection as recommended by U.S. EPA (1990c). If analyses of semivolatile compounds will not be performed within the recommended 7-day holding time, freezing of the samples at -20°C is advised. Holding times for frozen samples have not been established by the U.S. EPA. A general guideline of a maximum of 6 months would be consistent with that for water samples (U.S. EPA, 1987a).

Resectioning tissue samples for chemical analyses should only be conducted with noncontaminating tools under "clean room" conditions. It is recommended that whole tissue samples not be frozen prior to resection if analyses will be conducted only on selected tissues because freezing may cause internal organs to rupture and contaminate other tissues. A separate set of utensils for removing outer tissue and for resectioning tissue for analysis is highly recommended. Incision troughs are subject to contamination and should not be included in the analysis.

TABLE B10-6.
SUMMARY OF SAMPLE COLLECTION AND PREPARATION QA/QC REQUIREMENTS FOR ORGANIC COMPOUNDS

Variable	Sample Size[a]	Container[b]	Preservation	Maximum Holding Time
Tissues (After Resection)				
Semivolatiles	25 g	G,T	Freeze	6 months[c]
Volatiles	5 g	G,T	Freeze	14 days[c]

[a] Recommended field sample sizes for one laboratory analysis. If additional laboratory analyses are required (i.e., replicates). The field sample size should be adjusted accordingly.

[b] G = Glass, A = Wrapped in aluminum foil, placed in watertight plastic bags, T = PTFE (Teflon).

[c] This is a suggested holding time. No U.S. EPA criteria exist for the preservation of this variable.

Laboratory Analyses

Laboratory performance as measured by method QA/QC protocols should be used to evaluate and select appropriate analytical methods. Changes to laboratory protocols should only be considered if new protocols meet established performance criteria. Suggested QA/QC protocols and analytical performance criteria are discussed below.

QA/QC reports should describe the results of quantitative QA/QC analyses, as well as other elements critical to the laboratory analyses of water, sediments, and tissues to ensure proper interpretation of the results. It is recommended that these reports be recorded and stored in a database for future reference.

Field QA/QC Checks – Travel blanks will indicate whether any contamination was introduced by reagents in the field or during shipping of samples. Rinsate blanks are designed to verify the absence of contamination carried over from one sample to another due to inadequate cleaning of field equipment.

Field splits treated and identified as separate samples, may be sent to the same laboratory for analysis or one sample sent to a "reference" laboratory for comparison. In addition, standard reference material may also be placed in a sample container at the time of collection and sent "blind" to the laboratory.

Instrument QA/QC Checks – Calibration standards should be analyzed at the beginning of sample analysis, and should be verified at the end of each 12-hr shift during which analyses are performed (U.S. EPA, 1987b). The concentrations of calibration standards should bracket the expected sample concentrations, sample dilutions or sample handling modifications (i.e., reduced sample size).

Method QA/QC Checks – Analysis of method blanks should be conducted to demonstrate the absence of contamination from sampling or sample handling in the laboratory. At least one method blank must be included with each batch of samples and should constitute at least 5% of all samples analyzed.

Spike recovery analyses are required to assess method performance for the particular sample matrix. Spike recoveries serve as an indication of analytical accuracy, whereas analysis of standard reference materials measure extraction efficiency. Recommended control limits include 75-125 percent recovery for spikes, and 80-120 percent recovery for the analysis of standard reference materials.

Replicates must be analyzed to monitor the precision of laboratory analyses. A minimum of 5% of the analyses should be laboratory replicates. The control limits are ±20 percent relative percent difference for duplicates. Triplicates should be analyzed on one of every 20 samples or on one sample per batch if less than 20 samples are analyzed.

Tables B10-7 and B10-8 provide a brief summary of QA/QC for laboratory analyses. A discussion of detection limits may be found in QA/QC Considerations in the Water Column Chemistry chapter (Section B2.4).

B10.5 Statistical Design Considerations

Statistical strategies may mitigate the high costs of collecting sufficient tissue biomass. See also Statistical Design Considerations: Composite Sampling, Statistical Power, and Power-Cost Analysis (Section B.3).

B10.6 Use of Data

Results of the bioaccumulation analyses can be used to establish spatial and temporal trends in the bioaccumulation of toxicants of selected estuarine fish and macroinvertebrates. Geographic maps of these trends would provide a survey of trends throughout the estuary.

In addition, monitoring studies might identify existing and potential problem areas for fish and macroinvertebrate contamination. Human health risk, as well as environmental risk assessment calculations may also be determined from data collected from these studies. The information may be used to develop management strategies for commercial and recreational fishing.

B10.7 Summary and Recommendations

Rationale

- Presence of toxics in waters and sediments of estuaries can have adverse ecological and human health effects

- Monitoring the accumulation of chemical residues in tissues of estuarine organisms will provide information essential in relating the presence of selected contaminants in estuarine waters and sediment to its transfer and accumulation in estuarine organisms

TABLE B10-7.
SUMMARY OF QUALITY CONTROL SAMPLE

Analysis Type	Recommended Frequency of Analysis[a]
Surrogate spikes	Required in every sample - minimum 3 neutral, 2 acid spikes, plus 1 spike for pesticide/PCB analyses, and 3 spikes for volatiles. Isotope dilution techniques (i.e., with all available labeled surrogates) is recommended for full scan analyses and to enable recovery corrections to be applied to data.
Method blank	One per extraction batch (semivolatile organics). One per extraction or one per 12 hour shift, which ever is most frequent (volatile organics)
Standard reference materials	<50 samples: one per set of samples submitted to lab >50 samples: one per 50 samples analyzed
Matrix spikes	Not required if complete isotope dilution technique used <20 samples: one per set of samples submitted to lab ≥20 samples: 5 percent of total number of samples.
Spiked method blanks	As many as required to establish confidence in method before analysis of samples (i.e., when using a method for the first time or after any method modification).
Analytical replicates	<20 samples: one per set of samples submitted to lab ≥20 samples: one triplicate and additional duplicates for a minimum of 5 percent total replication.
Field replicates	At the discretion of the project coordinator.

[a] Frequencies listed are minimums; some programs may require higher levels of effort.

TABLE B10-8.
SUMMARY OF WARNING AND CONTROL LIMITS FOR QUALITY CONTROL SAMPLE

Analysis Type	Recommended Warning Limit	Recommended Control Limit
Surrogate Spikes	10 percent recovery	(50 percent recovery)
Method Blank		
Phthalate, Acetone	30 percent of the analyte	5 µg total or 50 percent of the analyte
Other Organic Compounds	1 µg total or 5 percent of the analyte	2.5 µg total or 5 percent of the analyte
Standard Reference Materials	95 percent confidence interval	95 percent confidence interval for Certified Reference Material
Matrix Spikes	(50-65 percent recovery)	(50 percent recovery)
Spiked Method Blanks	(50-65 percent recovery)	(50 percent recovery)
Analytical Replicates	–	±100 percent coefficient of variation
Field Replicates	–	–
Ongoing Calibration		25 percent of initial calibration

- Monitoring program may provide information necessary to assess environmental and human health risks associated with measured levels of bioaccumulation

Monitoring Design Considerations

- It is recommended that consistent types of target species and tissues, data normalization, and location and timing of sample collection be implemented to allow for comparisons among studies

- Target species should possess the following characteristics:
  - high bioaccumulation potential for selected contaminants of concern
  - metabolic regulation of selected contaminants should be weak or absent
  - abundant, temporally and spatially, to allow for adequate sampling
  - large enough to provide adequate amounts of tissue for analysis
  - sessile, or sedentary in nature to assure bioaccumulation is representative of the study area
  - easily collected

- Analyses should be conducted on a number of different target species to ensure sensitive measures of bioaccumulation for the contaminants of concern

- Caged indicator species
  - method allows for control of initial temporal and spatial variation of individuals and/or biomass
  - method allows for the use of specific age, size and/or genetic stocks

- Indigenous species
  - results obtained will relate directly to those species which may be impacted
  - no possibility of loss of holding apparatus and test organisms

- Target tissues
  - fish liver or macroinvertebrate hepatopancreas analyses may be used to estimate the range of contaminants being assimilated

- muscle tissue analyses are appropriate for human exposure assessments and quantitative health risk determinations
- whole body analyses ought to be conducted when predators consume the whole body of the target organism

- TOC/AVS/Lipid Normalization
  - recommend normalization to allow comparisons of chemical residue concentrations in tissues between locations, individuals, and tissue type – normalizing the data will depend upon the objectives of the individual monitoring program
  - differences in lipid analysis techniques will result in differences in lipid detected – caution in comparing data between analytical methods

- Time of Sampling
  - hydrophobic contaminants are mobilized and transported to the egg during oogenesis
  - timing of sampling should be based on biological cycles which influence an organism's susceptibility to bioaccumulate
  - it is recommended that target species be sampled when tissue contamination concentrations are expected to be at their highest levels

Existing Analytical Methods

- It is recommended that consistent types of analytical protocols be implemented to allow for comparisons among studies

- Metals/Metalloids
  - GFAAS is the recommended method for the detection of metals and metalloids (U.S. EPA, 1987a)
  - cold vapor AAS is the recommended protocol for mercury detection

- Organics
  - GC/MS in conjunction with isotope dilution is recommended for the detection of semi-volatile organic compounds
  - vacuum super-cooled trap in conjunction with a purge and trap device is recommended for the detection of volatile organics (Hiatt, 1981)

- isotope dilution option is recommended as it provides reliable recovery data for each analyte (U.S. EPA, 1986a)

QA/QC Considerations

- Reports delineating the essential elements of the bioaccumulation component of the program should be included with the quantitative QA/QC analyses

Statistical Design Considerations

- Six individuals comprising each of five replicate composites should be adequate to detect a treatment difference equal to 100 percent of the overall mean among treatments

Use of Data

- Establish spatial and temporal trends in the bioaccumulation of toxicants of selected estuarine fish and macroinvertebrates
- Identify existing and potential problem areas for fish and macroinvertebrate contamination
- Supply data which can be used to calculate the human health risk of consuming estuarine fish and shellfish

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B11.0 Bacterial and Viral Pathogens

Monitoring for pathogenic microorganisms is currently conducted by state environmental and human health agencies in shellfish harvesting areas and bathing beaches. National Pollution Discharge Elimination System (NPDES) pathogen monitoring programs may be underway, at selected locations in the estuary, in order to assess both the condition of water in the vicinity of discharges and surrounding areas and to assess relative pathogen contributions from these permitted effluent discharges.

The National Shellfish Sanitation Program (NSSP) has been established as a joint effort between the Food and Drug Administration (FDA), state agencies, and the shellfish industry to set forth guidelines for the management of state shellfish programs. As part of the NSSP, FDA provides technical assistance to states in studying specific pollution problems; provides data to establish closure levels for shellfish harvesting; conducts applied research on various contaminants to assist in developing standards and criteria; and evaluates the effectiveness of state shellfish sanitary control programs. In addition, since 1966, data have been compiled periodically by FDA and the National Oceanic and Atmospheric Administration (NOAA) on classification by states of coastal and estuarine waters with regard to suitability for shellfishing activities. In addition to classifying their waters as to their suitability as shellfish harvesting areas, states also issue beach closures. These closures are typically based on water quality criteria developed by the Federal government.

B11.1 Rationale

Human pathogens found in the estuarine environment include viruses and bacteria. In the United States, viruses and bacteria are the most important human pathogens, both in terms of the number of organisms released to the environment and in the severity of the diseases they cause (OTA, 1987).

Humans can be exposed to pathogens by direct contact with contaminated waters (e.g., swimming, surfing, diving) and/or indirectly through ingestion of contaminated food (e.g., molluscan shellfish). The preponderance of evidence indicates that the etiologic agent for waterborne outbreaks of acute gastroenteritis (AGI) are the Norwalk-like viruses (Kaplan *et al.*, 1982). Hepatitis A has been linked to the consumption of raw or partially cooked molluscan shellfish (Feingold, 1973; CDC, 1979; Ohara *et al.*, 1983). Bacteria responsible for typhoid and cholera are known to be water- and seafood-borne. These pathogens can enter the estuarine environment through the discharge of raw sewage, wastewater effluent from sewage treatment plants, failing septic tanks, and the dumping of sewage sludge (OTA, 1987).

Monitoring of human pathogens provides information essential in relating the presence of pathogens in marine waters and shellfish to outbreaks of disease. Monitoring data

may be used to identify potential sources of pathogens. The assessment of pathogen contamination should be a component of a monitoring program where pathogens may present a risk to human health and economic vitality of an estuary.

B11.2 Monitoring Design Considerations

Water Column Sampling

Bacteria are not uniformly distributed throughout the water column (Gameson, 1983); bacterial abundances, several orders of magnitude greater than underlying waters, can be found in a thin microlayer on the surface of the water (Hardy, 1982). If feasible, it is recommended that samples of this microlayer be collected; separate samples of underlying waters should also be taken. However, standardized methods for sampling this microlayer have not been established. If it is not feasible to sample both the microlayer and underlying waters, the "scoop" method should be used to ensure that the surface microlayer is sampled (U.S. EPA, 1978).

Water samples for bacterial analyses are frequently collected using sterilized plastic bags (e.g., Whirl-pak) or screw-cap wide-mouthed bottles. Several depths may be sampled during one cast and/or replicate samples may be collected at a particular depth using a Kemmerer or Niskin sampler (U.S. EPA, 1978). Any device which collects water samples in unsterilized tubes should not be used for collecting bacteriological samples without first obtaining data that support its use. Pumps may be used to sample large volumes of the water column (U.S. EPA, 1978).

Sentinel Organisms

Analyses of sentinel shellfish tissue (e.g., mussels and oysters) offer several advantages:

- they concentrate pathogens; they may be useful for viral analyses since viruses are often present in low numbers
- they provide a means of temporally integrating water quality conditions
- they may be direct measures of human exposure to pathogens (i.e., consumption of contaminated food)
- they may be deployed and maintained in a number of diverse locales

However, the disadvantages of sentinel organisms include:

- the cost of transplanting organisms
- the possibility of loss of the cage/buoy system
- species may not be tolerant of all test site conditions (e.g., low salinity, high turbidity)

It is essential that monitoring design elements be standardized to allow for comparisons among estuarine studies. The selection of a standard sentinel species is recommended; interspecies differences would not allow comparisons of body burdens.

B11.3 Analytical Methods Considerations

Many pathogens can be present in wastes, contaminated media, and infected organisms. Human pathogens are of concern in estuarine waters because they are:

- associated with major debilitating diseases (e.g., hepatitis, cholera)
- infectious at low doses
- resistant to environmental stress
- not readily enumerated due to low numbers

Table B11-1 provides examples of pathogenic organisms known to cause adverse human health effects. Due to difficulties in culturing and identifying pathogens that occur in very low abundances, standardized protocols have not been developed for their identification and enumeration. The alternative to directly identifying pathogens of concern has been the enumeration of bacteria which are indicators of human waste contamination or indicators of human illness.

Indicators of Human Health Risks

Due to the inability to enumerate pathogens of concern, indicators of human pathogen densities have been used in order to assess human health risks. Indicator organisms useful in predicting infectious disease rates should have the following characteristics:

- high abundances should be consistently found in human fecal wastes

TABLE B11-1.
MICROORGANISMS RESPONSIBLE FOR CAUSING ADVERSE HUMAN HEALTH EFFECTS* (NOAA, 1988)

Disease	Pathogenic Organism	Seafood Source
Hepatitis	Hepatitis A virus	Raw oysters Steamed and raw clams Cockles
	Non-A and non-B hepatitis	Raw molluscan shellfish
Gastroenteritis	*Aeromonas hydrophilia* and *Plesiomonas shigelloides*	Shellfish
	Vibrio mimicus	Raw oysters
	Vibrio parahaemiolyticus	Clams and snails Raw oysters Crab Shrimp Lobster
	Vibrio vulnificus	Raw oysters
	Vibrio cholera O group	Raw oysters boiled shrimp, boiled crab
	Vibrio cholera, Non-O group 1	Raw oysters
	Norwalk virus	Raw oysters Raw clams
	Small round structured virus	Raw oysters
	Campylobacter jejuni	Raw clams

* Includes naturally occurring microorganisms as well as microorganisms associated with pollution.

- should not have significant extra-human fecal sources
- must provide temporally and spatially reliable and accurate appraisals of the pathogen of concern

Currently, there is no consensus on which indicator organism, if any, is specific for human feces.

Coliform Bacteria - For decades, the concentration of coliform bacteria, either total or fecal coliform, has been considered a reliable indicator of the presence and densities of pathogens. The use of total coliform bacterial criteria to protect human health from disease associated with contaminated water is widespread. Furthermore, total coliform concentrations have the advantage of providing a basis for comparison with historical data (U.S. EPA, 1988).

However, these indicators and their corresponding water quality standards have not been related to incidences of disease through epidemiological studies. Controversy exists on the efficacy of using coliform bacteria to predict the presence of other types of pathogens (Pederson, 1980). In fact, recent studies indicate that fecal coliform bacteria may not be a reliable indicator for predicting the risks associated with direct exposures to pathogens in the marine environment (Cabelli *et al.*, 1979 and 1982). Fecal coliforms are not pathogenic, and are less resistant to environmental stress compared to many pathogens (Borrego *et al.*, 1983). Furthermore, fecal coliform bacteria are not specific to mammalian fecal pollution. The lack of this specificity prompted the development of methods for enumerating fecal coliform bacteria specific to mammalian fecal pollution (e.g., *Escherichia coli*). However, with respect to recreational water quality criteria, the EPA has not recommended the use of *E. coli* for marine and estuarine waters.

Enterococci - Enterococci are streptococcus bacteria indigenous to the intestines of warm-blooded animals. Cabelli *et al.* (1983) found that the densities of enterococci were highly correlated with the incidence of gastrointestinal symptoms (GI) among swimmers; reported swimming-associated GI symptoms were poorly correlated with fecal coliform densities. Enterococci also have the following advantageous characteristics:

- tolerant to high salinity and are of particular value in the analysis of estuarine and marine waters

- taxonomic identifications are relatively simple and can reveal the kinds of mammalian pollution (e.g., humans, livestock)

- genetic fingerprinting techniques have been developed which can link these bacteria in the environment to specific sources of contamination

State-of-the-art genetic fingerprinting techniques are very costly and require further study in order to assess their reliability. The EPA has adopted enterococci as an indicator of microbiological water quality for recreational marine waters.

Viruses - Viruses are being recognized as major etiologic agents for many outbreaks of human illness as a result of swimming in contaminated water and consumption of contaminated seafood. Viruses are excreted only by infected individuals; they are not normal flora in the intestinal tract. Traditional indicators of microbial contaminants appear to be inadequate for predicting human health risks associated with both consuming molluscan shellfish and swimming in waters that contain sewage associated viruses. However, examination of water for enteric viruses is not recommended at this time, except in special circumstances, due to limitations of methodologies. Even state-of-the-art methods for concentrating viruses from water still are being researched and continue to be modified and improved. None of the available virus detection methods have been tested adequately with representatives from all of the virus groups of public health importance. In addition, some of these methods require expensive equipment and materials for sample processing and all virus assay and identification procedures require expensive cell culture and related virology laboratory facilities.

Laboratory Techniques

It should also be noted that no single procedure is adequate to isolate all microorganisms from water, and the presence of one microorganism does not signify the presence or absence of any other (Table B11-2). A more detailed description of analytical methods for a number of pathogens is presented in Standards Methods for the Examination of Water and Wastewater (APHA, 1989).

Fecal Bacteria - Two standard methods are presented here for the detection of fecal bacteria: membrane filter procedure, and multiple-tube fermentation procedure.

The membrane filter (MF) technique involves sample filtration followed by direct plating for detection and enumeration of coliform bacterial densities. The MF technique can be used to test relatively large volumes of samples, and yields numerical results more rapidly than the multiple-tube procedure. The statistical reliability of the

TABLE B11-2.
LABORATORY PROCEDURES FOR BACTERIAL INDICATORS

Test Organisms	Laboratory Procedures		
	Water	Sediment	Tissue
Fecal coliform bacteria	MPN tubes using A-I broth (APHA, 1989) (fecal coliform bacteria/100 ml)	MPN tubes using A-I broth (APHA, 1989) (fecal coliform bacteria/100 ml)	MPN tubes using EC broth (APHA, 1989) (fecal coliform bacteria/100 ml)
Fecal coliform bacteria/*E. coli*	mTEC (DuFour *et al.*, 1981) (E. coli/100 ml)	–	–
Enterococci	mE (Levin *et al.*, 1975)[a] (enterococci/100 ml)	–	–
C. perfringens[b]	mCP by membrane filtration (Bisson and Cabelli, 1979) (*C. perfringens*/100 ml)	mCP by membrane filtration (Emerson and Cabelli, 1982) (*C. perfringens*/100 ml)	MPN tubes using iron milk (St. John *et al.*, 1982) (*C. perfringens*/ 100 ml)

[a] This method is a tedious process, EPA Region II and the State of New Jersey have developed a modified mE isolation technique.

[b] Two laboratory techniques are available for *C. perfringens*: mCP by membrane filtration for water (Bisson and Cabelli 1979) and sediment (Emerson and Cabelli 1982), and iron milk tube using MPN techniques (St. John *et al.*, 1982). The Bisson and Cabelli (1979) method is preferred.

MF technique is greater than the Most Probable Number (MPN) procedures (APHA, 1989). However, the MF technique has limitations, particularly in testing waters with high turbidity and noncoliform (background) bacteria. The MF technique can be used to measure bacterial densities of *Escherichia coli* (*E. coli*) and enterococci in ambient waters (U.S. EPA, 1985); the EPA has approved this technique for use in seawater.

The multiple-tube fermentation technique examines a series of fermentation tubes containing growth media which are inoculated with the appropriate decimal dilutions of water (multiples and submultiples of 10 ml), based on the probable coliform density. Formation of gas in any amount of time constitutes a preliminary positive for the presence of coliform bacteria; confirmation phase analyses are then conducted. The precision of the test depends on the number of tubes examined in each dilution.

Adequate mixing of the sample in order to yield a homogenous dispersion of bacteria in the test portions is critical to the accuracy of the test; clumping of bacteria will result in underestimations of actual bacterial density. This technique is commonly used in assessing bacterial levels in shellfish.

The most probably number (MPN) index is an index of the number of coliform bacteria that, most probably, would produce the results observed in the laboratory examination (APHA, 1989). Values for the MPN index can be obtained from standard tables based on the results of the multiple-tube fermentation technique or from Thomas' formula (APHA, 1989).

Viruses - Detecting viruses in estuarine waters requires collecting a representative water sample, concentrating viruses in the sample, and identifying and estimating abundances of these concentrated viruses. Difficulties in detecting virus abundances include:

- viruses are very small (20-100 nm)
- virus concentrations in waters are spatially and temporally variable, and typically low
- dissolved and suspended material in the sample interferes with accurate virus detection

Because virus concentrations may be very low, significant volumes of water may be required (e.g., on the order of tens to thousands of liters). Dose-response curves are lacking for most pathogens; however, as few as 10 to 100 bacteria are capable of inducing disease (OTA, 1987).

Three different techniques for concentrating and enumerating viruses are presented in Standard Methods (APHA, 1989):

- adsorption to and elution from microporous filters
- aluminum hydroxide adsorption and precipitation
- polyethylene glycol hydroextraction-dialysis

The adsorption and elution technique pressure-filters viruses on microporous filters and then elutes them from the filter in a small liquid volume. Generally, two types of filters are available: electronegative and electropositive filters. Currently, insufficient documentation on the efficacy of electropositive filters exists. Limitations to this technique include:

- clogging of adsorbent filter by suspended material
- dissolved colloid material may interfere by competing with viruses for adsorption sites on the filter
- viruses adsorbed to suspended material may be removed during suspended material clean-up procedures

Methods for recovering solids-associated viruses are found in Standard Methods (APHA, 1989). In spite of these limitations, the adsorption and elution technique remains the most promising technique for detecting viruses.

The aluminum hydroxide adsorption and precipitation, and the polyethylene glycol hydroextraction-dialysis techniques are used to reconcentrate viruses in proteinaceous and organic buffer eluates. These methods may be used to concentrate viruses in waters having high virus densities (e.g., wastewaters). They may also be used as a second-step concentration procedure following processing of large fluid volumes through microporous filters. However, these two techniques are impractical for primary processing of large fluid volumes (APHA, 1989).

New Techniques – Traditional techniques lack the ability to detect apparently viable, but non-culturable microorganisms. Recently developed monoclonal antibody and gene probe techniques permit the detection and enumeration of both culturable and non-culturable microorganisms. Since these methods have the ability to detect non-culturable organisms, they may serve as more precise techniques for monitoring microbiological water quality. Furthermore, these new techniques will allow direct monitoring of pathogens of concern; the promise of monitoring specific pathogens, such as *Salmonella*, *Shigella*, *Giardia*, or *Legionella* may soon be realized.

Sample Handling

Preservation and storage of water samples can introduce significant sources of error. Sample bottles must be resistant to sterilization procedures.

Samples should be refrigerated (1-4°C) during transport to a laboratory and analyzed within six hours of collection (U.S. EPA, 1978).

B11.4 QA/QC Considerations

It is recommended that sterile distilled water be transported to the field, transferred to a sample bottle, and processed routinely to ensure that samples were not contaminated during collection and transport. It is also recommended that ten percent of the samples be analyzed in duplicate. Furthermore, ten percent of the samples should be split and analyzed by two or more laboratories.

Intra-laboratory and inter-laboratory quality control practices should be documented and QA/QC reports should be available for inspection. Further recommended quality assurance guidelines for a microbiology laboratory are available (Inhorn, 1977; Bordner *et al.*, 1978) and are also discussed in Standard Methods (APHA, 1989).

B11.5 Statistical Design Considerations

Bacterial counts often are characterized as having a skewed distribution because of many low values and a few high ones (APHA, 1989). Application of parametric statistical techniques requires the assumption of symmetrical distributions such as the normal curve. An approximately normal distribution can be obtained from positively skewed data by converting numbers to their logarithms (APHA, 1989). Accordingly, the preferred statistic for measuring central tendency of microbiological data is the geometric mean.

Consideration of statistical strategies will mitigate the high costs of collecting and processing samples. Analyses of power-costs are necessary in selecting appropriate sample/replicate number, sample location and sampling frequency (Ferraro *et al.*, 1989). See also Statistical Design Considerations: Composite Sampling, Statistical Power, and Power-Cost Analysis (Section B.3).

B11.6 Data Use

The assessment of pathogen contamination should be a component of a monitoring program where pathogens may be present a risk to human health and the economic vitality of an estuary. Monitoring of human pathogens provides information essential in relating the temporal and spatial distribution of infectious agents in estuarine waters and shellfish to the epidemiology of pathogens of concern (e.g., affected human populations, locations, and timing of the outbreak).

Furthermore, monitoring data can be used to identify discharges which may be significant sources of pathogens and to ensure that water quality standards are maintained. Monitoring data can also be used to verify fate and transport, human health risk assessment, and epidemiological modeling predictions.

B11.7 Summary and Recommendations

Rationale

- The objective is to detect and describe spatial and temporal changes in abundances of indicators of human health pathogens

- Monitoring indicators of human pathogens provides information essential in relating the presence of infectious agents in estuarine waters and shellfish to the incidence of disease outbreaks and potential sources of these agents.

Monitoring Design Considerations

- If feasible, samples of the surface microlayer should be collected separate from samples of underlying waters. If not feasible, samples containing both underlying and microlayer waters should be collected. It is highly recommended that consistent types of sampling protocols be implemented to allow for comparisons among studies

- Analysis of tissues of sentinel organisms (e.g., mussels and oysters) confers the following advantages:
  - they concentrate pathogens
  - they provide a means of temporally integrating water quality conditions
  - they may be deployed and maintained in a number of locales

Analytical Methods Considerations

- Indicators of human pathogens should have the following characteristics:
  - be consistently found in high abundance in human fecal wastes
  - should not have significant extra-human fecal sources
  - must provide temporally and spatially reliable and accurate appraisals of the pathogen of concern

- Fecal Coliform Bacteria Densities
  - may not be a reliable indicator for predicting the risk associated with direct exposures to pathogens
  - this measure provides a means to compare to historical data
  - Laboratory analyses include membrane filter technique and multiple-tube fermentation technique

- Enterococci Densities
  - are highly correlated with the incidence of gastrointestinal symptoms
  - taxonomic identifications are relatively simple and can reveal the kinds of mammalian pollution
  - state-of-the-art genetic fingerprinting techniques can link bacteria in the estuary to specific sources; however these techniques are very costly and require further study in order to assess their reliability
  - Laboratory analyses include membrane filter technique

- Viral Densities
  - recognized as major etiologic agents for many outbreaks of human illness
  - examination of water samples for enteric viruses is not recommended due to limitations of methodologies
  - Laboratory analyses include absorption to and elution from microporous filters, aluminum hydroxide absorption-precipitation, and polyethylene glycol hydroextraction-dialysis

- Monoclonal antibodies and gene probes show promise in detecting and enumerating both culturable and non-culturable microorganisms

QA/QC Considerations

- Sterile distilled water should be transported and transferred to sample bottles in order to assess contamination during collection and transport

- Ten percent of the samples should be analyzed in duplicate

- Ten percent should be split and analyzed by two or more laboratories

Statistical Design Considerations

- Preferred statistic for measuring central tendency of microbiological data is the geometric mean

Use of Data

- Provide essential information in order to assess threats to human health
- Establishes temporal and spatial trends in pathogen densities and identifies potential relationships between the presence of the indicator and incidence of human illness
- Provides data which can be used to verify fate and transport, human health risk, and epidemiology models

B11.8 Literature Cited and References

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