Combined Work/Quality Assurance Project Plan (CW/QAPP)

for

Fish and Shellfish Monitoring: 1995-97

Tasks 21, 22, 23, 24 and 25
MWRA Harbor and Outfall Monitoring Project

submitted to

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1.0 PROJECT NAME

Fish and Shellfish Monitoring
Tasks 21, 22, 23, 24, and 25
MWRA Harbor and Outfall Monitoring Project

2.0 PROJECT REQUESTED BY

Massachusetts Water Resources Authority

3.0 DATE OF REQUEST

November 2, 1994

4.0 DATE OF PROJECT INITIATION

November 9, 1994

5.0 PROJECT MANAGEMENT

Dr. Michael Connor, MWRA Director of Environmental Quality Department
Dr. Michael Mickelson, MWRA Harbor and Outfall Monitoring Project Manager
Mr. Maury Hall, MWRA Project Area Manager (Bioaccumulation Survey)
Mr. Ken Keay, MWRA Harbor and Outfall Monitoring Deputy Project Manager and Project Area Manager (Flounder and Lobster Surveys)

Dr. James Blake, ENSR Project Manager for Harbor and Outfall Monitoring
Dr. James Bowen, ENSR Technical Director for Harbor and Outfall Monitoring
Dr. David F. Mitchell, ENSR Fish and Shellfish Monitoring Area Manager
Dr. Phillip Downey, Inchcape, Principal Investigator
Dr. Michael Moore, Woods Hole Institution, Principal Investigator

6.0 QUALITY ASSURANCE MANAGEMENT

Ms. Wendy Leo, MWRA EM and MS Manager
Ms. Debra McGrath, ENSR Project QA Director

7.0 PROJECT DESCRIPTION

7.1 Objective and Scope

The Massachusetts Water Resources Authority (MWRA) is continuing a long-term biomonitoring program for fish and shellfish (MWRA, 1991) for the MWRA effluent outfall that will be located in Massachusetts Bay (see Figure 1). The goal of the biomonitoring is to provide baseline data that may be used to assess potential environmental impact of effluent discharge into Massachusetts Bay and to evaluate compliance with the NPDES discharge permit.

The overall objective of the fish and shellfish monitoring is to define the baseline condition of winter flounder, lobster, and mussel health in terms of the presence of disease (external and internal), and organic and inorganic (metal) contaminant concentrations in the liver (winter flounder), hepatopancreas (lobster), and edible tissue (winter flounder, lobster and mussel). To help determine the body burden of toxic substances and to assess the physiological status of winter flounder *(Pleuronectes americanus)* and lobster *(Homarus americanus),* one survey per species will be conducted in Boston Harbor, Massachusetts Bay, and Cape Cod Bay (hereafter: Boston Harbor and the Bays) during 1995, 1996 and 1997 to collect specimens for analysis. To determine body burden and physiological status of blue mussel *(Mytilus edulis),* arrays of mussels will be deployed in Boston Harbor and Massachusetts Bay and collected during 1995, 1996 and 1997. With a sound baseline characterization of the health of winter flounder, lobster, and mussel in Boston Harbor and the Bays, it should be possible to observe potential changes resulting from the relocation of the outfall discharge. Specific objectives for each of the five tasks included in this program are described in Sections 7.1.1 through 7.1.5.

This Combined Work/Quality Assurance Project Plan (CW/QAPP) presents the organization, objectives, functional activities, and specific quality assurance (QA) and quality control (QC) activities associated with the Fish and Shellfish Monitoring that will be conducted under Tasks 21 through 25 of the MWRA Harbor and Outfall Monitoring Program (Contract S186). This document also describes the specific protocols that will be followed for sampling, sample handling and storage, chain of custody, and laboratory and field analyses. The CW/QAPP was prepared in accordance with EPA guidance documents on CW/QAPP

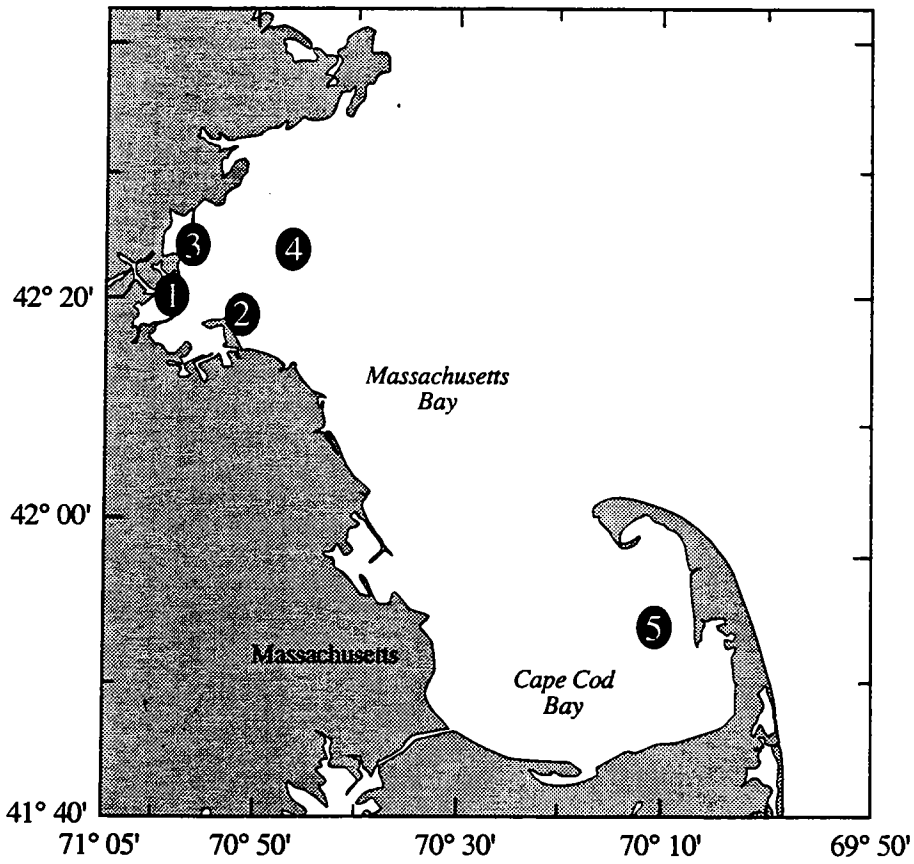

FIGURE 1-1
Fish and Shellfish Monitoring Stations

preparation (EPA 1984, 1988) and is based on the CW/QAPP produced previously under a contract between MWRA and Battelle Ocean Sciences (Hillman, 1993). Separate survey plans developed for each survey will supplement the CW/QAPP. The survey plans will provide the operational details required to conduct each survey, and will describe participating staff, detailed schedule, and specific equipment.

7.1.1 Flounder Survey (Task 21)

The objective of the survey is to obtain specimens of winter flounder *(Pleuronectes americanus)* from five sampling sites in Boston Harbor and the Bays for gross examination, histology, aging, and chemical analyses of tissue to determine baseline health and tissue burden of contaminants. Specimens will be collected during surveys conducted in April 1995, 1996 and 1997.

7.1.2 Lobster Survey (Task 22)

The objective of the survey is to obtain specimens of lobster *(Homarus americanus)* from three sampling sites in Boston Harbor and the Bays for gross examination and chemical analyses of tissues to determine baseline health and tissue burden of contaminants. Specimens will be collected during surveys conducted in July 1995, 1996 and 1997.

7.1.3 Mussel Bioaccumulation Survey (Task 23)

The objectives of the survey are to obtain, deploy, and recover blue mussels *(Mytilus edulis)* for determination of biological condition and short-term accumulation of anthropogenic contaminants in soft tissues. Specimens will be collected during surveys conducted in June-August 1995, 1996, and 1997.

7.1.4 Tissue Chemical Analyses (Task 24)

The objective of tissue chemical analyses is to determine the baseline body burdens of toxic substances by measuring the concentrations of organic and inorganic (metal) substances in flounder, lobster, and mussel collected under Tasks 21-23.

7.1.5 Flounder Histological and Mussel Condition Analysis (Task 25)

The objective of the histological analysis is to assess the health of the flounder populations in Boston Harbor and the Bays by performing microscopic examinations of tissue sections of the flounders' livers

collected under Task 21. The baseline health of the various flounder populations will be determined based on the prevalence and severity of various lesions in the tissues. The objective of the aging analysis is to determine the age composition of the specimens that are examined for histological and chemical analysis.

The objective of the mussel condition analysis is to determine the physiological and reproductive status of the mussel population in Boston Harbor and the Bays.

7.2 Data Usage

7.2.1 Tissue Chemical Analyses (Task 24)

Chemistry data will be used to determine the concentrations of organic and inorganic (metals) contaminants in flounder liver, lobster hepatopancreas, and flounder, lobster, and mussel edible tissue (fillet; claw and tail meat; and meat respectively) prior to the relocation of the existing discharge of any effluent. The contaminant concentrations will also be related to any histological lesions observed or indices of physiological condition.

Results of these analyses will be used to evaluate the impact of discharging effluent into Massachusetts Bay, the recovery of Boston Harbor, and the impact of relocating the effluent on fish and shellfish health.

7.2.2 Flounder Histological and Mussel Condition Analysis (Task 25)

Histological data will be used to assess the health of the flounder populations in the Boston Harbor and Bays areas prior to the relocation of the existing effluent discharge. Age data will be used to determine the age of the adult population of winter flounder in the sampling areas prior to the discharge of the effluent. Mussel condition (growth and reproductive) will be used to assess the health of mussel populations in the Boston Harbor and Bay areas prior to the relocation of the existing effluent discharge.

7.3 Technical Approach

7.3.1 Flounder Surveys (Tasks 21)

A three-day flounder survey will be conducted annually during April 1995, 1996, and 1997. The general technical approach to conducting the flounder survey includes the following components:

- Preparation and submittal of a Survey Plan for each survey;
- Collection of winter flounder from the sites in Boston Harbor and the Bays; and
- Preparation and submittal of Survey Report for each survey

A Survey Plan will be prepared for each of the three surveys. The Survey Plan will detail sampling dates, locations, vessel, personnel, any deviations from the CW/QAPP, and general survey operations. These plans will be delivered to MWRA no later than two weeks before the start of each survey.

ENSR will conduct three flounder surveys. Five sites will be sampled to collect winter flounder for histological and chemical analyses (Figure 1). Table 1 provides the sampling sites and locations although there may be differences of 1 km or more between collection sites and the indicated position due to the trawling operations. Figure 1 illustrates the sampling locations in Boston Harbor and the Bays.

At each of the five designated sampling sites otter-trawl tows will be conducted to collect 50 sexually mature (4-5 years old) winter flounder. Fifteen fish will be randomly selected for joint histological and chemical analysis. These fish will be maintained alive on-board and transported to Woods Hole for histological and chemical analysis. The remaining 35 fish will be killed at sea by cervical section and used for histological processing. The gonads of each flounder will be examined to determine sexual maturity. All specimens will be weighed, and standard and fork length will be determined. Scales will be taken from each specimen. On board, each flounder will be examined externally and their external condition will be noted. In addition, the liver will be removed and examined for grossly visible abnormalities. Chemical and histological analyses of liver and edible tissue samples will be conducted in the laboratory.

Within 30 days after each flounder survey, a Survey Report will be prepared and submitted to MWRA. Each report will contain information on winter flounder collection operations, maps of actual survey tracks for each day of the survey, number of flounder collected, number of fish collected, and current status and disposition of histological and chemical samples. The report will detail any problems encountered or departures from stated protocols and their potential impacts on the program.

7.3.2 Lobster Survey (Task 22)

A lobster survey will be conducted annually during mid-July 1995, 1996, and 1997. The general technical approach to conducting the lobster survey includes the following components;

TABLE 1

Sampling Sites and Locations for Flounder/Lobster/Mussel Surveys

		Location		Survey Type		
Station #	Sampling Site	Latitude	Longitude	Flounder	Lobster	Mussel
1	Deer Island Flats (Boston Harbor)	42°20.4'	70°58.4'	*	*	*
2	Off Nantasket Beach	42°17.6'	70°52.2'	*		
3	Broad Sound	42°24.4'	70°57.2'	*		
4	The site of the future effluent outfall	42°23.1'	70°49.3'	*	*	*
5	Eastern Cape Cod	42°56.2'	70°06.6'	*	*	
6	Boston Inner Harbor	42°21.5'	71°02.9'			*
7	Gloucester	TBD	TBD			R
8	Sandwich/Cape Cod	TBD	TBD			R

TBD = To be determined
* = Sampling Site for Survey
R = Reference Site for Mussel Bioaccumulation Survey

- Preparation and submittal of a Survey Plan for each survey;
- Collection of lobster from three sites in Boston Harbor and the Bays; and
- Preparation and submittal of a Survey Report for each survey.

A Survey Plan will be prepared for each of the three surveys. The Survey Plan will detail sampling dates, locations, vessel, personnel, any deviations from the CW/QAPP, and general survey operations.

ENSR will conduct three lobster surveys. Three sites will be sampled to collect lobster for chemical analyses. Table 1 provides the sampling sites and locations. Figure 1 illustrates the sampling locations in Boston Harbor and the Bays.

A string of 25 to 30 lobster pots will be set to collect 15 commercially harvestable lobsters at each site. Individual lobsters retained for analysis will be assigned a unique identification number to indicate date, time, and site of collection. Lobsters will be measured for carapace length and width and gender determined. Lobster specimens will be visually examined and the condition noted. Chemical analyses of the hepatopancreas and edible tissue samples will be conducted in the laboratory.

Within 30 days after each lobster survey, a Survey Report will be prepared and submitted to MWRA. Each report will contain information on lobster collection operations, the number of lobster collected, maps of the location of lobster pots, and current status and disposition of chemical samples. The report will detail any problems encountered or departures from stated protocols and their potential impacts on the program.

7.3.3 Mussel Bioaccumulation Survey (Task 23)

A mussel bioaccumulation survey will be conducted annually in June-August 1995, 1996 and 1997. The general technical approach to conducting each mussel survey includes the following components:

- Preparation and submittal of a Survey Plan for each survey;
- Deployment and retrieval of mussels from four sites in Boston Harbor and Massachusetts Bay; and
- Preparation and submittal of a Survey Report for each survey.

A Survey Plan will be prepared for each of the three surveys. The Survey Plan will detail sampling dates, locations, vessel, personnel, any deviations from the CW/QAPP, and general survey operations.

ENSR will conduct three mussel bioaccumulation surveys. Mussels will be deployed and retrieved at three sites plus baseline reference site(s). The collected mussels will be examined for biological condition and provide tissue samples for chemical analysis. Table 1 provides the sampling sites and locations although the exact location of the deployment may differ by 1 km (or more) from the indicated position. Figure 1 illustrates the sampling locations in Boston Harbor and Massachusetts Bay.

Mussels will be deployed in June in replicate arrays at three sites. These mussels will have previously been collected from the baseline reference sites. Mussels will be retrieved on two occasions (i.e., partial or mid-deployment period, and full deployment period) and examined for biological condition or frozen and set aside for chemical analysis.

Within 30 days after the final retrieval, a Survey Report will be prepared and submitted to MWRA. Each report will contain information on mussel deployment and collection operations, maps indicating locations of mussel arrays, number of mussel collected during mid-deployment and full deployment sampling, and current status and disposition of biological and chemical samples. The report will detail any problems encountered or departures from stated protocols and their potential impacts on the program.

7.3.4 Tissue Chemical Analysis (Task 24)

Chemical analysis will be performed on composite samples of flounder, lobster, and mussel tissue. Composite samples will be prepared through random selection of samples and homogenization of tissue. The following number of samples will be prepared and used for chemical analysis.

For flounder, three groups of five individual fish will be pooled from the 15 collected to create three pooled samples per site. Two tissue types (fillet, liver) are to be analyzed. This results in 30 pooled samples (3 pools x 5 sites x 2 tissue types). Chemical analyses will be performed on samples from three sites in 1995 and 1997, and for all sites in 1996. The additional flounder sample collected in 1995 and 1997 will be frozen and archived. The chemical analyses to be performed on sample and tissue types are indicated in Table 2.

For lobster, five commercially-catchable lobsters will be pooled from the 15 collected to create three pooled samples per site. Two tissue types are to be analyzed per site (claw or tail meat, hepatopancreas). This results in 18 pooled samples (3 pools x 3 sites x 2 tissue types). The chemical analyses to be performed on sample and tissue types are indicated in Table 2.

TABLE 2

Chemistry Analysis Parameters

Organism	Number/ Type of Samples	Parameters
Flounder	9/fillet*	Mercury PCB Chlorinated pesticides Lipids
Flounder	9/liver*	Trace metals PCB PAH Chlorinated pesticides Lipids
Lobster	9/meat	Mercury PCB Chlorinated pesticides Lipids
Lobster	9/hepatopancreas	Trace metals PCB PAH Chlorinated pesticides Lipids
Mussel	23/soft tissue	Mercury Lead PCB PAH Chlorinated pesticides Lipids
*15 samples during 1996 survey.		

For mussel, ten mussels will be pooled from the 50 collected to create five pooled samples per site except for the future outfall site where eight pooled samples will be created from 80 mussels. This results in 23 pooled samples (5 pools x 3 sites + 8 pools x 1 site). The chemical analyses to be performed on mussel samples are indicated in Table 2.

7.3.5 Flounder Histological and Mussel Condition Analysis (Task 25)

Fifty flounder will be selected from each of the five sampling sites for the suite of histological analyses. One section, 5 μm thick, from each of three transversely cut portions of livers from 50 flounder collected at each of five sites during each survey will be examined histologically. A total of 250 slides each containing three liver sections, will be prepared and examined each year (1995, 1996 and 1997). Lesions to be scored include hydropic vacuolation, macrophage aggregation, biliary duct proliferation, and neoplasia.

The age of each specimen will be determined by reading the number of annuli on a scale from that specimen.

Thirty mussels will be selected from each of the sites and analyzed for the following parameters: shell weight, shell length, viability status at recovery, total tissue (wet/dry weight) and gonadal tissue (wet/dry weight). From these analyses, two condition factors (gonadal condition index and total soft/shell condition index) will be calculated.

7.4 Monitoring Parameters and Collection Frequency

Table 2 summarizes the primary chemical parameters that will be measured for each organism or sample type (Task 24). Table 3 summarizes the parameters that will be measured in Task 25 for flounder histology. Table 4 summarizes the number of organisms and the types of analyses that will be conducted on samples collected from each station. Table 5 lists the specific analytes that will be measured.

8.0 PROJECT FISCAL INFORMATION

This project is being carried out under the Harbor and Outfall Monitoring contract (Contract No. S186) between MWRA and ENSR Consulting and Engineering.

TABLE 3

Histology Analysis Parameters

Organism	Number/Type of Samples	Parameters
Flounder	50/liver	Vacuolation Macrophage aggregation Biliary duct proliferation Neoplasia
Flounder	50/Scale	Age

TABLE 4

Field Samples and Measurements

Organism	Parameter	Numbers of Sampling Units Total[a]/Sample[b]	Container	Shipboard or Laboratory Processing/Preservation
Winter flounder	Chemistry - liver - edible tissue	50/3 50/3	Clean, labeled jar	Shipboard: Flounder are kept alive until processing or dissection Laboratory: Freeze, if not processed immediately
	Histology	50/50	Clean, labeled jar	Laboratory, Shipboard: 10% neutral buffered formalin
	Age (scales)	50/50	Age envelope	Shipboard: Clean mucous from sampling area of fish before taking scales
	Visual	50/50	N/A	Shipboard: Describe qualitatively
Lobster	Chemistry - hepatopancreas - edible tissue	15/3 15/3	Clean, labeled jar	Shipboard: Lobster are held in coolers with ice or immediately frozen Laboratory: Freeze, if not processed immediately
	Visual	15/15	N/A	Shipboard: Describe qualitatively
Mussel2	Chemistry - soft tissue	50/5 or 80/8[c]	Clean, labeled jar	Shipboard: Mussels place in coolers with ice Laboratory: Freeze if not processed immediately
	Biometrics - shell dimension - body wt (wet/dry) - gonad wt (wet/dry) - reproductive condition - sex	30/30	Clean, labeled container	Laboratory: Process immediately

[a]Total individual specimens collected per station.
[b]Total pooled (composite) samples to be analyzed per station.
[c]Total mussel number for LNB station only.

TABLE 5

Analytes Included in Tissue Chemistry Analyses

Trace Metals[a]
Ag Silver
Cd Cadmium
Cr Chromium
Cu Copper
Hg Mercury[b,d]
Ni Nickel
Pb Lead[d]
Zn Zinc
Polychlorinated biphenyls (PCBs)[c,d]
2,4,-Cl_2(8)
2,2′,5-Cl_3(18)
2,4,4′-Cl_3(28)
2,2′,3,5′-Cl_4(44)
2,2′,5,5′-Cl_4(52)
2,3′,4,4′-Cl_4(66)
3,3′,4,4′-Cl_4(77)
2,2′4,5,5′-Cl_5(101)
2,3,3′,4,4′-Cl_5(105)
2,3′,4,4′5-Cl_5(118)
3,3′,4,4′,5-Cl_5(126)
2,2′,3,3,4,4′-Cl_6(128)′
2,2′,3,4,4′,5-Cl_6(138)
2,2′4,4′,5,5′-Cl_6(153)
2,2′3,3,4,4′,5-Cl_7(170)
2,2′,3,4,4′,5,5′-Cl_7(180)
2,2′,3,4,5,5′,6-Cl_7(187)
2,2′,3,3′,4,4′,5,6-Cl_8(195)
2,2′,3,3′4,4′,5,5′,6-Cl_9(206)
Decachlorobiphenyl-Cl_{10}(209)
Polynuclear Aromatic Hydrocarbons (PAHs)[a,d]
Naphthalene
C_1-naphthalenes
C_2-naphthalenes
C_3-naphthalenes
Acenaphthylene
Acenaphthene
C_1-fluorenes
C_2-fluorenes
C_3-fluorenes
Phenanthrene
C_1-Phenanthrenes/anthracene
C_2-Phenanthrenes/anthracene
C_3-Phenanthrenes/anthracene
C_4-Phenanthrenes/anthracene
Polynuclear Aromatic Hydrocarbons (PAHs) (continued)
Dibenzothiophene
C_1-dibenzothiophenes
C_2-dibenzothiophenes
C_3-dibenzothiophenes
Fluoranthene
Pyrene
C_1-fluoranthenes/pyrene
Benzo[*a*]anthracene
Chrysene
C_1-chrysene
C_2-chrysene
C_3-chrysene
C_4-chrysene
Benzo[*b*]fluoranthene
Benzo[*k*]fluoranthene
Benzo[*a*]pyrene
Dibenzo[*a,h*]perylene
Indeno[1,2,3-*c,d*]pyrene
Perylene
Biphenyl
Benzo[*e*]pyrene
Dibenzofuran
Benzothiazole[e]
Pesticides[c,d]
Hexachlorobenzene
Lindane
Heptachlor
Endrin
Aldrin
Heptachlorepoxide
alpha-chlordane
trans-Nonachlor
Dieldrin
Mirex
2,4′-DDD
4,4′-DDD
2,4′-DDE
4,4′-DDE
2,4′-DDT
4,4′-DDT
DDMU[e]
Lipids[c,d]

[a] Flounder liver; lobster hepatopancreas
[b] Flounder and lobster edible tissue
[c] Flounder edible tissue and liver; lobster edible tissue and hepatopancreas
[d] Mussel soft tissue
[e] New analyte for indicated survey

9.0 SCHEDULE OF ACTIVITIES AND DELIVERABLES

The schedule of activities and deliverables for this project is tied to survey activities. Table 6 provides the 1995 - 1997 planned schedule for all survey plans, survey cruises, survey reports, and data reports required for Tasks 21, 22, 23, 24, and 25.

The deliverables for Tasks 21, 22, 23, 24, and 25 are (1) survey plans and survey reports for each of the three surveys, and (2) draft and final Annual Fish and Shellfish Reports for each survey. The due dates for the data reports are shown in Table 6.

10.0 PROJECT ORGANIZATION AND RESPONSIBILITIES

The project organization is shown in Figure 2. Dr. Michael Mickelson is the MWRA Project Manager. Mr. Maury Hall is the MWRA Project Area Manager for the Bioaccumulation Survey. Mr. Ken Keay is the Deputy Project Manager and serves as backup to both Mr. Mickelson and Mr. Hall. Mr. Keay is also the MWRA Project Area Manager for Flounder and Lobster Survey. They will be informed of all matters pertaining to work described in this CW/QAPP. Dr. James Blake is the ENSR Project Manager responsible for the overall performance of this project. Dr. David F. Mitchell is the ENSR Project Area Manager responsible for the conduct of the fish and shellfish monitoring tasks described in this CW/QAPP.

Dr. Michael Moore (WHOI) is the principal investigator for the Flounder Survey and histological analyses. Dr. Phillip Downey (Aquatec Inchcape) is the principal investigator for the mussel bioaccumulation survey and condition analysis. Laboratory analysis is coordinated through Dr. Gregg Douglas (Arthur D. Little) and Mr. Raveendra Ika (Envitec).

Ms. Therese Mounce is the Task Manager for the Flounder Survey (Task 21) and the Lobster Survey (Task 22). Mr. Mark Gerath is the Task Manager for the bioaccumulation survey (Task 23). Dr. Eric Butler is the Task Manager for chemical analyses (Task 24). Mr. Stephen Cibik is the Task Manager for the Flounder Histology/Mussel Condition Analysis (Task 25). The task managers are responsible for monitoring the day-to-day activities for their respective tasks and for communicating progress and problems to the Project Area Manager.

TABLE 6

Schedule of Deliverables

Task	Deliverable	Due Date
Flounder Survey (Task 21)	1995 Survey Plan 1995 Survey Cruise 1995 Survey Report 1996 Survey Plan 1996 Survey Cruise 1996 Survey Report 1997 Survey Plan 1997 Survey Cruise 1997 Survey Report	March 1995 April 1995 May 1995 March 1996 April 1996 May 1996 March 1997 April 1997 May 1997
Lobster Survey (Task 22)	1995 Survey Plan 1995 Survey Cruise 1995 Survey Report 1996 Survey Plan 1996 Survey Cruise 1996 Survey Report 1997 Survey Plan 1997 Survey Cruise 1997 Survey Report	June 1995 July 1995 August 1995 June 1996 July 1996 August 1996 June 1997 July 1997 August 1997
Bioaccumulation Study (Task 23)	1995 Survey Plan 1995 Survey Cruise 1995 Survey Report 1996 Survey Plan 1996 Survey Cruise 1996 Survey Report 1997 Survey Plan 1997 Survey Cruise 1997 Survey Report	May 1995 June-August 1995 September 1995 May 1996 June-August 1996 September 1996 May 1997 June-August 1997 September 1997
Tissue Chemical Analyses (Task 24)	1995 Tissue Chemistry Data Report 1996 Tissue Chemistry Data Report 1997 Tissue Chemistry Data Report	November 1995 November 1996 November 1997
Flounder Histology and Mussel Condition Analysis (Task 25)	1995 Histology Data Report 1995 Mussel Biological Condition Report 1996 Histology Data Report 1996 Mussel Biological Condition Report 1997 Histology Data Report 1997 Mussel Biological Condition Report	August 1995 October 1995 August 1996 October 1996 August 1997 October 1997

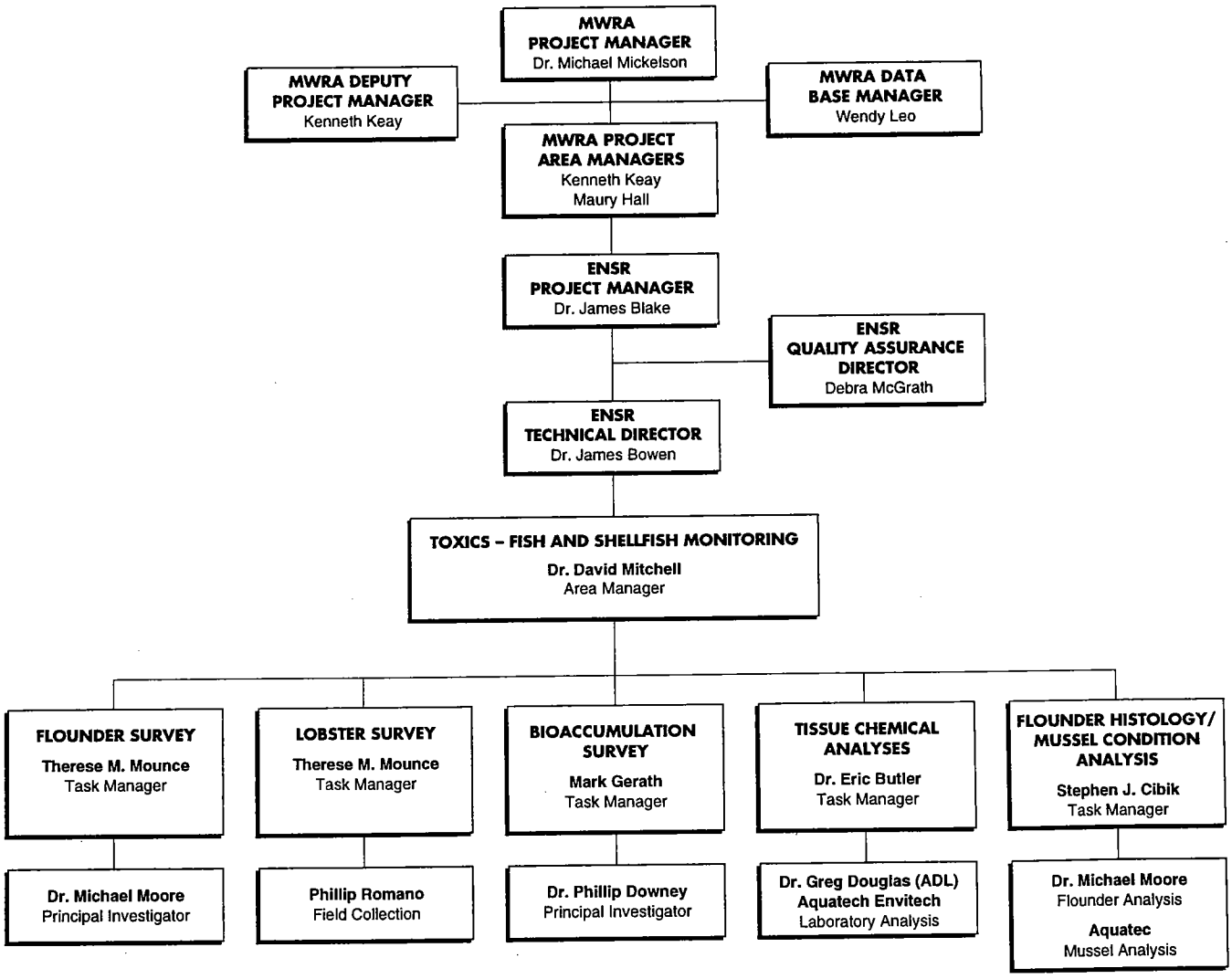

FIGURE 2
Fish and Shellfish Monitoring Team

M950548

Ms. Debra McGrath, Project QA Director, is responsible for administering the QA program for all technical activities conducted by ENSR.

11.0 DATA QUALITY REQUIREMENTS AND ASSESSMENTS

To ensure that all data generated during the conduct of Tasks 21, 22, 23, 24 and 25 are of known and acceptable quality, data will be examined in terms of the following characteristics:

Accuracy – the extent of agreement between the measured value and the true value
Precision – the extent of agreement among independent, similar, or related measurements
Completeness – measure of the amount of data acquired relative to the amount of data required to fulfill the objectives and statistical criteria for the intended use of the data
Comparability – the extent to which data from one study can be compared directly to similar studies
Representativeness – the extent to which sample locations and measurements represent true systems

11.1 Navigational Data

11.1.1 Precision and Accuracy

All sampling will be conducted within the geographical boundaries of the sampling sites (see Figure 1). During high winds or in the presence of strong water currents, the vessels will be positioned 300 m upstream of the status position at the start of the otter trawl. Deployment of lobster pots or mussel arrays should be within 300 m of target location. GPS/LORAN will be used to achieve the required accuracy. Section 12 provides details on relevant sampling procedures to ensure data quality and Section 14 discusses instrument calibration methods.

11.1.2 Completeness

A Northstar GPS navigation system or equivalent will be used to output navigation positions at beginning and end of a trawl deployment. The Principal Investigator or Chief Scientist will be responsible for recovery position.

If instrument malfunctions occur and operations are modified or suspended during any survey day, a decision on modification of activities for that survey will be made with consultation and agreement of MWRA, whenever possible.

11.1.3 Comparability

Latitude/longitude positions will be recorded by the Principal Investigator or Chief Scientist. These positions will be comparable to positions obtained by other tasks in the MWRA monitoring project as well as by other researchers that have used or are using GPS or corrected LORAN. The station locations are targets; sampling will be conducted within 300 m of the targets, according to the navigation display. Sampling objectives will be achieved with respect to positioning by this level of station accuracy.

The instrumentation and methods of data reduction that will be used during the fish and shellfish monitoring surveys are similar to the instrumentation routinely used by EPA, the National Oceanic and Atmospheric Administration (NOAA), and other research institutions working in Massachusetts Bay. Thus, the data should be consistent with and comparable to previous studies. During review and synthesis of the survey data, the results will be compared with the general ranges of data obtained from previous studies, including recent surveys conducted in 1992 - 1994.

11.1.4 Representativeness

The corrected latitude/longitude positions are representative of the actual vessel coordinates and survey track because position data are collected and reviewed at a frequency that ensures that the measured latitude/longitudes represent the actual vessel position.

11.2 Flounder Collection (Task 21)

At each station, 50 winter flounder specimens will be collected. Samples of liver will be taken from each specimen for histological and chemical analysis. Edible tissue will be taken from 15 flounder from each site for chemical analyses.

11.2.1 Accuracy

To ensure that specimens are correctly identified, fish keys, such as *Guide to Some Trawl-Caught Marine Fishes from Maine to Cape Hatteras, North America* (Flescher, 1980) and field guides will be used. The

guaranteed accuracy of the "Chatillon" fish scale is 50 g. The accuracy of the fish measuring board is 0.1 cm.

11.2.2 Precision

The precision of the weights of the fish will be enhanced by using a scale (Chatillon fish scale) with a maximum reading pointer (MRP) that retains the weight reading of the fish until another fish is put on the scale. If time allows, the first 15 specimens collected at each sampling site will be measured and weighed twice. If agreement between the length or weight measurements is within 1 cm or 0.05 kg, respectively, measurements will continue. If measurements or weights differ by more than the above-stated criteria, the specimen will be remeasured or weighed.

11.2.3 Completeness

The objective is to obtain 50 sexually mature specimens from each sampling site. Otter-trawl tows will be conducted until at least 50 specimens are collected at each sampling site. Sampling will be 100% complete when this is accomplished. In the event of sample loss or equipment malfunction, the Principal Investigator will determine the need for appropriate corrective action (e.g., resampling using a different otter trawl). The corrective action taken by the Principal Investigator will be recorded in the survey records. In the event of inadequate numbers of fish, three hours of bottom time will be the maximum effort expended at any one station.

11.2.4 Comparability

Winter flounder have been routinely used by other researchers working in Massachusetts Bay and similar environments to determine baseline health and tissue burden of contaminants. Collection of winter flounder that are sexually mature will allow a comparison of the health of adult specimens at each sampling site. The methods of collection and visual analyses are comparable to methods used by Dr. Michael Moore for previous studies on winter flounder from these sampling sites. Otter trawls have been used by the National Oceanic and Atmospheric Administration (NOAA) National Marine Fisheries Service (NMFS) and the Massachusetts Division of Marine Fisheries since the 1960s and 1970s, respectively, as a method to sample finfish. All otter-trawl tows will be conducted for approximately 30 minutes at a speed of 1.5 to 2 kn. The sampling design of this survey is comparable to the design of previous surveys.

11.2.5 Representativeness

The sampling sites represent previously sampled locations and represent the population of winter flounder that may be found within Massachusetts and Cape Cod Bays.

11.3 Lobster Collection (Task 22)

At each station, 15 lobster specimens will be collected. Samples of hepatopancreas and edible tissue will be taken from each specimen for chemical analysis.

11.3.1 Accuracy

The guaranteed accuracy of the lobster scale is 50 g. The accuracy of the fish measuring board is 0.1 cm. The accuracy of the calipers is 1 mm.

11.3.2 Precision

If time allows, the first two specimens collected at each sampling site will be weighed and measured twice. If agreement between the measurements (length or weight) is within 1 mm or 0.05 kg, respectively, measurements will continue. If measurements or weights differ by more than the above-stated criteria, the specimen will be remeasured or weighed.

To ensure that only commercially harvestable specimens are retained, all specimens will be measured using a lobster gauge and females will be inspected for the presence of eggs. Calipers will be used to measure specimens after it is determined that they are of harvestable size. Any specimens that have a carapace length < 3.25 inches (the minimum legal size) or that contain eggs will immediately be returned to the environment. These measurements and inspections will be made immediately upon capture to improve chances of survival. [Note: The measurements and inspections are identical with commercial practice.]

11.3.3 Completeness

The sampling objective is to obtain 15 commercially harvestable specimens representative of their location. Lobster pots will be set repeatedly at each sampling site until 15 lobsters of legal size, but without eggs, are collected. Lobster pots will be set with the assistance of an experienced commercial fishermen to

optimize baiting and deployment of traps. Completeness will be 100% when 15 lobsters are collected from each sampling site. In the event of sample loss or equipment malfunction, the ENSR Task Manager will determine the need for appropriate corrective action. The corrective action taken by the ENSR Task Manager will be recorded in the survey records.

11.3.4 Comparability

Lobster have been routinely used by other researchers working in Massachusetts Bay and similar environments to determine baseline health and tissue burden of contaminants. Lobster pots are routinely used by commercial fishermen to collect lobster. The sampling design of this survey is comparable to the design of previous surveys.

11.3.5 Representativeness

The sampling sites represent previously sampled locations and represent the population of lobsters that may be found within Massachusetts and Cape Cod Bays.

11.4 Mussel Collection (Task 23)

At each station 80 or 110 mussels will be collected from the deployed arrays. The mussels will be used for biological analyses (30 mussels) and chemical analyses (50 or 80 mussels).

11.4.1 Accuracy

Accuracy of mussel measurements is addressed under Task 25 (Section 11.6.1).

11.4.2 Precision

Precision of mussel measurements is addressed under Task 25 (Section 11.6.2).

11.4.3 Completeness

The deployment arrays will contain excess numbers of mussels at the start of the incubation and a portion of mussels will be retrieved midway in the incubation period. Completeness will be 100% after recovery of the 60 day deployment when 80 or 110 mussels are collected from each sampling site. In the event of array or sample loss, the Principal Investigator will determine the need for appropriate corrective action. The correction action taken by the Principal Investigator will be recorded in the survey records.

11.4.4 Comparability

The deployment and retrieval of caged mussels for short-term bioaccumulation is comparable to the approach used by the Mussel Watch project within the National Status and Trends Program of NOAA. In this case, mussels from one environment have been translocated to a different environment (sampling sites).

11.4.5 Representativeness

The sampling sites represent previously sampled locations and are representative of the expected short-term bioaccumulation conditions for mussels.

11.5 Tissue Chemical Analysis (Task 24)

11.5.1 Accuracy

Analytical accuracy will be evaluated based on percent recoveries of analytes in National Institute of Standards and Technology (NIST) standard reference materials (SRM), matrix spike samples, and the surrogate internal standards (SIS) that are added to every sample (organics only), as well as the results of the procedural blanks that will be analyzed with each batch of 15 to 20 field samples. [Note: NIST SRM is certified only for PAHs with reference ranges for other contaminants.] The QC samples will be analyzed at the following frequencies:

Organics:

- Every batch of samples will contain an SRM sample or certified control material.
- Each batch of samples will contain a matrix spike and matrix spike duplicate sample.

Metals:

- Every batch of samples will contain a field or laboratory duplicate sample.
- Each batch of samples will contain an SRM or certified control material.

Deviations from the above analytical scheme will require approval by the ENSR Task Leader and will be reported with the analytical data. All QC data will be reported with the sample data. The accuracy goal for organics and metals will be 70-130% recovery for SRMs for each individual analyte when concentrations are at least 10 times the analytical detection limits. The percent recovery goal for matrix spike samples will be 50-150%. Percent recovery will be calculated as shown in Section 15.2.3. Method detection limits (MDL) for analytes of interest have been calculated and are presented in Table 7 (PCB/Pesticides), Table 8 (PAH), and Table 9 (Metals).

All field samples, blanks, and matrix QC samples processed for organics analysis will be spiked with the appropriate SIS before extraction. Quantification of the SIS will be based on the recovery internal standards (RIS) added to the final extract just before instrumental analysis. Acceptable SIS recovery range will be 50%-150% (one PAH may exceed). It is considered acceptable if one of the PAH surrogate internal standards lies outside this range as long as the others are within the acceptable range. Because samples are quantified relative to the recovery of the SIS which is added before extraction, any loss of analytes during processing should be corrected by a comparable loss of the SIS. Therefore, recoveries of less than 50% may be considered acceptable. Each sample showing low recoveries will be individually examined by the laboratory manager or laboratory task leader to determine the necessity of reextraction or reanalysis and the proposed action discussed with the ENSR Task Leader. All corrective actions will be documented. When a sample does not meet the acceptance criteria and is not reanalyzed, the justification for this decision will be documented. QC exceedences will be reported to MWRA in the QA/QC Corrective Action Log.

Quality control criteria for accuracy are presented in Table 10.

11.5.2 Precision

Analytical precision will be determined using the concentrations of duplicate samples (matrix spikes for organics samples, field or laboratory duplicates for metals samples), with percent differences between duplicate analyses serving as a measure of precision. The goal for relative percent difference (RPD) for

TABLE 7

Polychlorinated Biphenyls/Pesticides Method Detection Limits for Tissues

Analyte	MDL (ng/g dry wt.)	Analyte	MDL (ng/g dry wt.)
CL_2(8)	2.76	2,4-DDD	0.58
Hexachlorobenzene	0.56	CL_5(118)	2.37
Lindane	1.02	4,4-DDD	1.01
CL_3(18)	1.59	2,4-DDT	0.89
CL_3(28)	0.83	CL_6(153)	2.10
Heptachlor	1.09	CL_5(105)	0.46
CL_4(52)	1.43	4,4-DDT	2.10
Aldrin	0.66	CL_6(138)	0.65
CL_4(44)	1.16	CL_5(126)	0.98
Heptachlorepoxide	0.96	CL_7(187)	0.67
CL_4(66)	0.97	CL_6(128)	1.23
2,4-DDE	1.09	CL_7(180)	1.21
CL_5(101)	1.54	Mirex	0.55
cis-Chlordane	1.16	CL_7(170)	1.1
trans-Nonachlor	1.01	CL_8(195)	0.49
Dieldrin	0.82	CL_9(206)	0.68
4,4-DDE	2.25	CL_{10}(209)	0.69
CL_4(77)	1.21	DDMU	2.25*

*Estimated value. Actual value will be reported after sample analysis.

TABLE 8

Polynuclear Aromatic Hydrocarbons Method Detection Limits[a] for Tissues

Analyte	MDL (ng/g dry wt.)	Analyte	MDL (ng/g dry wt.)
Naphthalene	10.85	Benzo[*b*]fluoranthene	9.75
Biphenyl	8.76	Benzo[*k*]fluoranthene	4.56
Acenaphthylene	10.02	Benzo[*e*]pyrene	4.27
Acenaphthene	3.80	Benzo[*a*]pyrene	3.63
Fluorene	6.39	Perylene	5.14
Phenanthrene	7.10	Indeno[1,2,3-*c,d*]pyrene	7.94
Anthracene	5.45	Dibenz[*a,h*]anthracene	7.16
Fluoranthene	9.45	Benzo[*g,h,i*]perylene	6.68
Pyrene	6.80	Dibenzofuran	6.39
Benz[*a*]anthracene	8.63	Dibenzothiophene	6.39
Chrysene	9.87	Benzothiozole	10.85[b]

[a]Alkylated PAH will be assigned corresponding parent PAH Method Detection Limit.
[b]Estimated value. Actual value will be reported after sample analysis.

TABLE 9

Metals Method Detection Limits for Tissues (Processed Mass 0.5 g dry weight)

Compound	MDL (µg/g dry wt.)	Compound	MDL (µg/g dry wt.)
Ag	0.025	Hg	0.025
Cd	0.025	Ni	0.5
Cr	.10	Pb	0.1
Cu	0.25	Zn	0.1
Fe	0.5		

MS/MSD samples is 30%. The RPD for laboratory duplicates is 20% for both individual analytes and the average of all analytes. The RPD will be calculated by the formula given in Section 15.2.

Quality control criteria for precision are presented in Table 10.

11.5.3 Completeness

The completeness of chemical analyses will be ensured by comparing the chain-of-custody forms received by the laboratory with the list of samples analyzed. Samples will be analyzed for the parameters listed in Table 2.

Completeness of chemical analyses will depend directly upon the amount of sample available. A minimum of 5 g (wet weight) of tissue is normally necessary to perform all of the required analyses. If inadequate tissue biomass is available, analyses may be conducted on lower weights (with potentially higher MDLs); subject to approval by MWRA Project Area Manager. Three pools of liver samples will be prepared. The number of samples composited for each pool will be based on the results of the histopathological analysis. If the volume of the combined livers is such that three pools are not at least 5 g, then the MWRA will be contacted prior to sample analyses for guidance on how to pool the samples. One-hundred percent of the samples collected for tissue chemistry analysis are expected to be analyzed, either individually or as composites.

11.5.4 Comparability

SRM when processed and analyzed with samples will quantify the comparability characteristic for laboratory measurements.

The data generated for this project will be directly comparable to data generated for the NS&T Mussel Watch project because the same analytical protocols are being used. In addition, all data developed for this project must be demonstrated to be comparable to data generated by other laboratories.

Additionally, the methods used by earlier MWRA studies (1989-1994) are directly comparable to the methods used for this project.

TABLE 10

Data Quality Objectives (Precision and Accuracy) for Organics and Metals Samples

QC Sample Type and Frequency	Data Quality Objective	Corrective Action[1]
Procedural Blank (Organics): 1/batch	<5*MDL	Reextraction, reanalysis, and/or blank subtraction
Method Blank (Metals): 1/batch	≈IDL	Blank subtraction
SRM Organics (PAH)*: 1/batch Metals: 1/batch	70-130% recovery	Reextraction, reanalysis, and/or blank subtraction
MS/MSD Organics: 1 set/batch	50-15% recovery ≤ 30% RPD	Report results
Duplicates Metals: 1 set per batch	Average % CV: 20% individual analyte 20% average of all analytes	
SIS (Organics): Every sample	50-150% recovery (one PAH SIS may exceed)	Results examined; possible reextraction or reanalysis.
Calibrations: Initial	Organics: ±30% RSD individual analyte ±15% RSD average of all analytes Metals: ±10% of true value .997 calibration correlation (r) (.99 for CVAAS)	Reanalysis
Check	Organics: ±30% RSD individual analyte ±20% RSD average of all analytes Metals: 10% of true value	Remedial maintenance, new initial calibration, sample reanalysis

[1] All corrective actions will be documented. Deviations from the stated corrective action will be justified.

* NIST SRM 1974A values are available only for PAH. It is anticipated that this reference material will be certified within the timeframe of the CW/QAPP. Consensus values are available for PCB and pesticides.

11.5.5 Representativeness

Not applicable

11.6 Flounder Histological and Mussel Condition Analysis (Task 25)

Histological observations of tissue abnormalities and scores assigned to these abnormalities are subjective based on the opinion of the pathologist reading the slides. Terms such as precision and accuracy, as they are defined in this document, are not applicable.

11.6.1 Accuracy

Scales and otoliths will be read by NMFS scientists that are experienced in aging winter flounder. A percentage of the scales will be reread to verify age determinations.

Mussel shell dimensions will be determined with a Vernier caliper which is accurate to 0.1 mm. Total soft tissue, gonadal tissue, and non-gonadal tissue will be measured on an electronic balance accurate to 0.01 g wet weight.

11.6.2 Precision

Not applicable

11.6.3 Completeness

For sufficient data for the statistical analyses needed to assess the health of the flounder populations, and to make intersite comparisons of the lesion prevalence, lesion scores from three slides from each of 50 flounder livers from each site will be calculated.

Large numbers of mussels are deployed in arrays which should provide sufficient mussels for biological (30) and chemical (50 or 80) analyses. An early (i.e., 40 day) retrieval is conducted to insure sufficient material is available for each site.

11.6.4 Comparability

The lesions scored, the method of scoring, and the data reduction and analyses will be similar to what has been done over the two previous years. Scales will be read as a courtesy by NMFS scientists that have aged winter flounder during the previous studies. Several slides will be studied with Dr. Robert Hillman to assure that observations are comparable to those made during studies conducted previously.

The determination of the shell dimensions, body and gonadal weight and reproductive condition of the mussels will be conducted by the same project team responsible for similar surveys the past three years. Dr. Phillip Downey will continue to be Principal Investigator.

11.6.5 Representativeness

Not applicable

12.0 SAMPLING AND ANALYTICAL PROCEDURES

Methods for collection and analysis of samples are described in the following sections.

12.1 Navigation

Vessel positioning during otter trawling and lobstering operations will be accomplished with a GPS navigation system. The GPS receiver has six dedicated channels and is capable of locking onto six different satellites at one time. This ensures strong signal reception and accurate and reliable positioning with 2-s updates. GPS and LORAN data will be recorded and both systems ground truthed at dockside.

Navigation data will be recorded to document survey activities. During otter-trawl towing or lobster-pot deployment operations, position fixes will be recorded. Position fixes will be made during discrete sampling events such as triggering the start of the otter-trawl towing or deploying a lobster trap.

12.2 Winter Flounder Collection and Processing

Winter flounder specimens will be collected and processed as described in the sections that follow.

12.2.1 Collection

1. A commercial otter trawl will be towed by a commercial dragger beginning at the site center of each sampling site listed in Table 1. The tows will be conducted for 15-30 minutes at a speed of 1.5 to 2 kn in a direction parallel to lobster-pot trawls in the area to avoid interaction with lobster pots. Tows will be conducted until at least 50 specimens have been collected at each sampling site. At the start and completion of each tow, the time and vessel position will be recorded.

2. At the completion of each tow, the otter trawl will be brought on board using a mechanical winch and the contents of the trawl unloaded onto the aft deck of the vessel. It may be necessary to conduct more than one otter-trawl tow at a sampling site if the required number of specimens (50) are not collected during the first tow. If the required number of flounder is not collected after six 30 minute tows and two 30 minute tows at an appropriate adjacent site, collections at that site will be terminated for the survey period.

3. All specimens will be sorted by species. However, only winter flounder will be retained; other species will be returned to the environment.

4. The following procedures will be followed to ensure that only >30 cm, sexually mature specimens are retained for analysis:

   (a) The fork length of specimens will be determined by measuring the length from the most anterior part of the fish to the tip of the median caudal fin rays. A measuring board will be used to obtain lengths.
   (b) Any specimens <30 cm (fork length) will be discarded because of the high probability (>50%) that they are sexually immature.
   (c) The gonads of the specimen will be inspected to determine sexual maturity based on the following criteria: immature fish have blue-gray gonads; mature females have pink, elongated gonads; mature males have white, triangular gonads. Any specimens that are not sexually mature will be discarded.
   (d) Specimens that are retained (50 from each site) will be weighed on a Chatillon fish scale and the weight recorded. The standard length will be determined by

measuring the length from the tip of the upper jaw to the posterior end of the hypural bone. The total length will also be measured.

5. The weight and lengths as well as other information will be recorded on the flounder sample collection log (Figure 3). A bar-coded label will be attached to the log.

12.2.2 Tissue Sample Processing

Processing will be conducted in the laboratory for the 15 fish for tissue chemistry analysis and histology and on board the collection vessel for the 35 fish for histology analyses.

Fish for Tissue Chemistry. Because it is unlikely that contaminant-free conditions will be found on board the vessel used for flounder collection, the fish used for chemical analysis will be returned to the laboratory for organ dissection. Of the 50 flounder collected from each site for histopathological analysis, 15 fish of the proper size will be designated for tissue chemical analysis. On the ship, standard and fork lengths, weights, and observations on external signs of disease will be determined and recorded on the log (see below for details). Age structures will also be removed (see below for details). The fish will be kept alive, identified by site-specific fin clips, until they are returned to the laboratory.

The flounder tissues will be removed in the laboratory under contaminant-free conditions. Tissue processing will be conducted in a laminar flow hood. Using a pre-cleaned (i.e., rinsed with 10% HCL, Milli-Q (18 megohm) water, acetone, DCM, and hexane) titanium knife, the fillets (muscle) will be removed from the flounder and the skin will be removed from the fillet. Composites will be made from 5 individual fish using approximately equal masses of top and bottom tissue. Each composite will be placed in a sample container clearly identified with a bar-coded label containing the unique sample identifier. At least one homogenization blank will be carried out for each batch to account for sample contamination during the homogenization process. For the blank sample, a known quantity of (about 100 ml) Milli-Q water will be transferred to the blender, blended for two minutes, and decanted into an acid-cleaned jar (the jars designated for sample homogenate collection). A subsample of water used for blank will also be collected in an HDPE bottle. Following processing of livers for histology analysis (see below), the homogenized tissue and liver samples will be frozen and stored.

Fifteen livers will be selected for chemical analysis. Following the processing for histology analysis, the livers will be divided randomly into three equal (by weight) pools. The Principal Investigator will provide the chemistry lab with the sample identifications of the livers for each pool.

Figure 3. Sample Collection Log — Winter Flounder

Specimen No.	Total Length (cm)	Standard Length (cm)	Weight (gm)	Sex (M/F)	Liver Color[a]	Fin-Rot Score[b]	Gross Lesions (describe)	Gross Score[c]	Samples Taken (check)
Study_____ Year_____ Event_____ Station_____ Visit_____ Sample #_____									liver: chem____ hist____ scales___ tissue___
Study_____ Year_____ Event_____ Station_____ Visit_____ Sample #_____									liver: chem____ hist____ scales___ tissue___
Study_____ Year_____ Event_____ Station_____ Visit_____ Sample #_____									liver: chem____ hist____ scales___ tissue___

Entered by:_____ **Date:**_____

[a]Liver Color Codes: Y-yellow; YB-yellow brown; B-brown; DB-dark brown; G-green
[b]Fin-Rot Score Codes: 0 - 4 (absent to extreme)
[c]Gross Score Codes: 0 - 4 (absent - extreme)

Fish for Histology Analyses. The following steps refer to the 35 fish that will be processed on board the vessel.

1. The remaining 35 fish from each site will be processed for histology analyses immediately, before proceeding to the next sampling site. The fish will be killed by means of a cervical section prior to processing.

2. On board, each flounder will be examined for external evidence of disease and notes will be recorded on the log.

3. Age structures (i.e., scales) will be collected from specimens >30 cm on board the vessel. Scales will be removed after first removing any mucus, debris, and epidermis from the dorsum of the caudal peduncle by wiping in the direction of the tail with a blunt-edged table knife. Scales will be collected from the cleaned area by applying quick, firm, scraping motions in the direction of the head. Scales will be placed in the labeled age-sample envelope by inserting the knife between the liner of the sample envelope and scraping off the scales.

4. The livers will be removed and examined for visible gross abnormalities. They will be preserved in 10% neutral buffered formalin for histological analysis. Liver samples from each fish will be placed in a separate clearly labeled sample container.

12.3 Lobster Collection and Processing

Lobster specimens will be collected and processed according to the procedures described in the sections below.

12.3.1 Collection

1. A lobster-pot trawl will be deployed up to three days within the geographical boundaries of each sampling site. The pots will be deployed in a direction parallel to other pots in the area. When the pots are deployed and retrieved, the time and vessel position will be recorded.

2. Upon retrieval, lobster pots will be brought alongside the vessel to the gunnel (side railing). Specimens will be removed and measured on the gunnel. No specimens will be brought on board the vessel until it is determined that they have a carapace length of at least 3.25 in (legal length). In addition, any specimens that are of legal length but also contain eggs (berried) will be returned to the environment immediately.

3. Fifteen specimens retained for processing will be placed in a flow-through seawater tank where they will be kept alive until transported to the laboratory for processing.

4. **Alternative Collection Method** - Following visual observation and documentation of the location of source lobster pots (within 2 km of target site), up to 15 legal-sized lobsters may be obtained from commercial lobstermen for a sampling site. Specimens will be processed as described below.

12.3.2 Lobster Processing

Lobsters will be collected from three sites. The lobster claws will be banded with coded claw bands to identify each site, and the claw band codes will be documented on the collection logs.

Carapace length will be determined by measuring the distance from the tip of the rostrum to the posterior edge of the median uropod with calipers. Measurements will be recorded to the nearest millimeter. Specimen weight will be recorded to the nearest gram. Specimens will be visually examined for the presence and severity of gross external abnormalities, such as black gill disease, shell erosion, and parasites. Data for each specimen will be recorded on lobster sample collection logs (Figure 4). A bar-coded or conventional label will be attached to the log and indicate site, project survey type, sample identification number, date and time, and sampler's initials. The hepatopancreas will be removed and frozen for chemical analysis. The tail and claw meat (edible tissue) will be stored frozen in the shells until processed in the laboratory. Samples will be placed in sample containers that are clearly identified with a bar-coded or conventional label containing the information described above.

The samples of hepatopancreas and edible tissue from 5 lobsters will be randomly divided into three equal (by weight) pools. The ENSR Task Leader will provide the chemistry lab with the sample identifications of the various tissues for each pool.

Figure 4. Sample Collection Log — Lobster

Specimen No.	Carapace Length (mm)	Weight (gm)	Sex (M/F)	Black gill[a]	Shell erosion[a]	Parasites[a]	External tumors[a]	Samples Taken (check)
Study_____ Year_____ Event_____ Station_____ Visit_____ Sample #_____								liver____ tissue ___
Study_____ Year_____ Event_____ Station_____ Visit_____ Sample #_____								liver____ tissue ___
Study_____ Year_____ Event_____ Station_____ Visit_____ Sample #_____								liver ___ tissue ___

Entered by:_____ Date:_____

[a]Codes: 0 - 4 (absent - extreme)

12.4 Mussel Bioaccumulation Survey

Mussel specimens will be deployed and retrieved according to the procedures described in the sections below.

12.4.1 Mussel Deployment

In June, mussels will be collected for testing purposes from the reference sites in Gloucester, MA and elsewhere, as appropriate. Mussels will be harvested during low tide and individually checked for length. Mussels which have a total average length of 55-65 mm will be used in the deployment. Fifty mussels will be randomly distributed to plastic cages for deployment as an array (i.e., set of cages) in sufficient number to provide the necessary biological material. A subsample of 80 mussels will be randomly selected and set aside for pre-deployment biological and chemical analyses.

Mussel array systems will be deployed at three locations. The locations of the arrays will be recorded.

12.4.2 Mussel Collection

After approximately 40 days, up to one half of the mussels from each array will be recovered to provide biological material in the event of a failure of the 60-day collection. Thirty mussels for biological parameters and 50 live mussels for chemical analyses (80 for analysis at outfall site) will be randomly selected. The mussels for chemical analysis will be placed in a clean container and frozen.

The amount of biofouling of the arrays will be assessed at 40 days. If necessary, arrays will be retrieved, cleaned, and re-deployed at the site.

At the end of 60 days, the remaining mussels will be collected. Thirty mussels will be randomly selected for biological analyses and 50 (or 80) will be selected for chemical analysis. The mussels for chemical analysis will be placed in a clean container and frozen.

12.4.3 Mussel Processing

A random subsample of mussels (50-80 depending on the station) will be selected from the predeployment mussels and from each of the three stations' 60-day mussel harvest. Replicate chemical analysis sample will be prepared as composite of ten mussels. Each individual mussel will be cleaned of attached material, all byssal threads removed, and all soft tissue including fluids placed directly into an amber 500-ml I-Chem Certified clean bottle. Mussel composite samples will be prepared for chemical analyses by dissection of each of the 10 mussels using disposable Teflon-coated stainless steel blades rinsed with methanol and deionized water prior to use. The Principal Investigator will provide the chemistry lab with the sample identifiers for the composite mussel samples.

In the event that sufficient mussels are not retrieved at 60 days, the MWRA Project Area Manager will be immediately notified. Following consultation of the Manager and ENSR, a revised approach to mussel analyses shall be determined.

12.5 Tissue Chemical Analyses (Task 24)

Table 2 summarizes the analyses of tissue samples collected under Tasks 21-23. Three pools each of flounder samples (liver and edible tissue) and lobster samples (hepatopancreas and edible tissue) and five or eight pools of mussel soft tissue will be analyzed (see Sections 12.2 and 12.3 for details on pooling). Samples assigned to each specific pool will be homogenized together prior to conducting analyses. The chemical analytes of interest for Task 24 are listed in Table 5.

12.5.1 Organic Analyses

Tissue samples will be serially extracted for PAH, chlorinated pesticides, and PCB. Briefly, an aliquot of homogenized tissue will be serially extracted with dichloromethane (DCM) and sodium sulfate using a Teckmar tissumizer. An aliquot of the original sample will also be taken for dry weight determination. The sample will be weighed in a Teflon extraction jar, spiked with the appropriate surrogate internal standards, sodium sulfate and solvent will be added, and the sample macerated for 2 minutes and centrifuged. The solvent extract will be decanted into an Erlenmeyer flask. After each extraction (total of two homogenizations and a third shake by hand), the centrifuged solvent will be combined in the flask. A 10-mL aliquot of the combined extracts will be removed for lipid weight determination, and sodium sulfate will be added to the extract in the flask. After approximately 30 minutes, the contents of the Erlenmeyer will be processed through an alumina column; because of the potentially high lipid content

of the liver and hepatopancreas samples, this step may be repeated, or samples may be pre-treated prior to extraction (e.g., KOH digestion). Any additional sample manipulations will be documented in the laboratory preparation records. The eluate from the alumina column(s) will be concentrated to 900 µL using a Kuderna-Danish apparatus and nitrogen evaporation techniques. The concentrated extract will be further cleaned using a high-performance liquid chromatographic (HPLC) gel-permeation technique. This procedure will remove common contaminants, including lipids, that interfere with instrumental analysis. The post-HPLC extract will be concentrated to approximately 500 µL under nitrogen gas and the recovery internal standards will be added to quantify extraction efficiency. The flounder liver, lobster hepatopancreas, or mussel tissue final extract will be split for analysis, one half remaining in DCM for PAH analysis, and the other half solvent-exchanged with isooctane for PCB and pesticide analysis. The entire final extract of flounder, lobster, or mussel edible tissue will be solvent-exchanged with isooctane for PCB and pesticide analysis.

Sample extracts will be analyzed for PAH compounds in the selected-ion-monitoring (SIM) mode by gas chromatography/mass spectrometry (GC/MS) using a modification of EPA Method 8270. The modifications are (1) operating the mass spectrometer in the SIM mode and (2) tuning the mass spectrometer with PFTBA. Pesticides and PCB congeners will be analyzed and quantified using gas chromatography/electron capture detection (GC/ECD) using EPA Method 8080, modified to include additional analytes and a second column for qualitative confirmation.

12.5.2 Metal Analyses

Tissue and liver samples will be digested using an aqua-regia microwave digestion procedure. Briefly, approximately 0.75 g of wet tissue or 0.3 g of dry tissue will be weighed into the teflon inserts of Parr Bombs. By the addition of 3 ml of superpure aqua-regia, the sample will be turned into a slurry. The bombs will be sealed and heated for three minutes. The bombs will be cooled, another 2 ml of superpure aqua-regia added and heated for two minutes. The digestate will be diluted to a final volume of 50 ml and transferred to a precleaned 125 ml polyethylene bottle. The digestate will be analyzed for silver (Ag), cadmium (Cd), chromium (Cr), nickel (Ni), copper (Cu), lead (Pb), and zinc (Zn) on ICP-MS with matrix matched external standards, corrected with the internal standard method. The EPA method for total recoverable mercury (EPA Method 245.1) will be modified to achieve lower detection limits. Mercury will be analyzed using a flow injection cold vapor technique with atomic absorption detection following preconcentration on gold amalgam as described by McIntosh et al., (1993).

12.6 Flounder Histological and Mussel Condition Analysis (Task 25)

Flounder histology and mussel biological analyses will follow the procedures described in the section below.

12.6.1 Flounder Histology

Livers of 50 flounder will be examined for histological analysis. The age of each flounder will be determined by NMFS scientists through analysis of growth rings (annuli) on the scales removed during the conduct of the Flounder Collection (Task 21) as described in CW/QAPP Section 12.2.2.

Transverse sections of flounder livers fixed as part of Tissue Sample Processing (see CW/QAPP Section 12.2.2.) will be removed from the buffered formalin after at least 24 hours, rinsed in running tap water, dehydrated through a series of ethanols, cleared in xylene, and embedded in paraffin. Paraffin-embedded material will be sectioned on a rotary microtome at a thickness of 5 μm. Each block will be sectioned at one level, resulting in one slide per fish and a total of 250 slides per year. The sections will be stained in hematoxylin and eosin.

Each slide will be examined under bright-field illumination at 25x, 100x, and 200x to quantify the presence and extent of:

- Three types of vacuolation (centrotubular, tubular hydropic, and focal hydropic)
- Macrophage aggregation
- Biliary duct proliferation
- Neoplasia

The severity of each lesion will be rated on a scale of 0 to 4, where: 0 = absent; 1 = minor; 2 = moderate; 3 = severe; and 4 = extreme. For each lesion and each fish, a histopathological index will then be calculated as a mean of scores from three slices on one slide.

12.6.2 Mussel Biological Condition

For biological analyses, a random subsample of 30 mussels will be selected from the predeployment mussels and from each of the three stations' 60-day collection. Mussels for biological analyses will be processed to obtain total shell length, total wet weight and reproductive condition.

In the laboratory, each mussel will be cleaned of attached material (barnacles, byssal threads, etc.). The total shell length (umbo to distal portion of valve gape) will be measured with a Vernier caliper (to the nearest 0.1mm). The total soft tissue, gonadal tissue, and the non-gonadal tissue weights will be measured on an electronic balance to the nearest 0.01g wet weight.

Each mussel will be opened by slicing the adductor mussels between the valves with a microtome blade. The gill tissue will be drawn back to expose the gonad and macerated gonad tissue will be examined under a compound microscope for the sex and sexual maturity.

13.0 SAMPLE CUSTODY

13.1 Navigation Data

Field custody of navigation data will be the responsibility of each task's Principal Investigator or ENSR Task Manager. This navigation data, including survey ID, date, time, trawl number, and vessel position at start and completion of each sampling event, will be recorded in the survey logbook.

13.2 Flounder, Lobster, and Mussel Samples

Before the field surveys, a checklist of all samples to be collected in the field will be prepared. To track samples from collection to analyses, protocol codes will be established based on the species, sample type, and analyses. Table 11 presents the protocols that will be used to identify flounder, lobster and mussel samples, composite samples (i.e., pooled samples), or sub-samples (e.g., liver, tissue) collected for Tasks 21 - 23.

The field sampler will be personally responsible for the care and custody of the samples form the time they are collected until they are transferred or dispatched properly. As few people as possible should handle the samples. Sample labels and chain-of-custody forms will be provided by ENSR.

All sample containers will be labeled with a unique identifier as described below and an MWRA sample identification number which will be used when reporting data to MWRA.

Samples will be identified by a unique five character sample ID. The first character will designate the study (P = flounder, L = lobster, M = mussel). The next two characters will be numbers designating the year, e.g., '95'. The next two characters will be a number designating the event number. For example

TABLE 11

Sampling Protocols for Flounder/Lobster/Mussel Surveys

Station	Flounder			Lobster		Mussel	
	Liver	Edible Tissue	Scales/ Otoliths	Hepatopancreas	Edible Tissue	Whole Mussel	Soft Tissue
01	FLC, FLH	FTC	FSA	LHC	LTC	MBA	MTC
02	FLC, FLH	FTC	FSA	NA	NA	MBA	MTC
03	FLC, FLH	FTC	FSA	NA	NA	MBA	MTC
04	FLC, FLH	FTC	FSA	LHC	LTC	MBA	MTC
05	FLC, FLH	FTC	FSA	LHC	LTC	MBA	MTC

Key: FLC = Flounder liver chemistry
FLH = Flounder liver histology
FTC = Flounder tissue chemistry
FSA = Flounder scale age
LHC = Lobster hepatopancreas chemistry
LTC = Lobster tissue chemistry
MBA = Mussel biological analyses
MTC = Mussel tissue chemistry
NA = Not applicable

P951 = Flounder sample taken in 1995 during first survey. Each specimen will be assigned a unique identification number which includes the sampling station, station visit (e.g., trawl or array), sample and bottle number. For example P95111045LH3 represents the third histological section from the 45th flounder taken at Station 1 during the first survey and visit of 1995. The sample number for composite samples will be "000". The field records will document the individual samples that are included in each composite.

Based on the types of samples that will be collected, chain-of-custody forms will be generated for each sample type to track samples from collection to laboratory. Figures 5a - 5f present examples of chain-of-custody forms that will be used. Manual entries will be recorded in indelible ink in the data section of the chain-of-custody only if the bar-coded label is destroyed or damaged. Each completed chain-of-custody will be signed and dated by the staff member entering the information. The chain-of-custody includes fields for entering the project name, sample location, sample type designation, alphanumeric sample codes (including sample ID), and pertinent information about each sample collection and general comments. This same information will be printed on barcoded labels. The barcode contains the sample ID. One label will be attached to the sample log form, one label will be attached to each sample container, and another label will be attached to the sample's accompanying chain-of-custody form.

Samples will be accompanied by a properly completed chain-of-custody form. When transferring the possession of samples, the individuals relinquishing and receiving will sign, date, and note the time on the record. This record documents the transfer of custody of samples from the sampler, to another person, to the laboratory, or to/from a secure storage location. Samples will be packaged properly for shipment and dispatched to the laboratory for analysis with a separate signed custody record enclosed in each sample cooler. ENSR will keep a copy of the chain-of-custody form, and will ensure that a copy accompanies the samples during transport. The original chain-of-custody form will be submitted to ENSR's database manager and maintained as part of the project files.

If a third party (i.e., commercial courier) is used to transfer the samples, chain-of-custody seals will be required. Coolers will be locked or secured with strapping tape and sealed with custody seals by the sample collectors. The preferred procedure is to attach a custody seal to the front right and back left of the cooler. The cooler will be taped closed with fiberglass tape covering the chain-of-custody seals. Commercial couriers will not be required to sign the chain-of-custody forms. However, the airbill will be maintained as part of the custody records.

<table>
<tr><td colspan="6">CHAIN-OF-CUSTODY RECORD</td></tr>
<tr><td colspan="6">For: Flounder Scales</td></tr>
<tr><td colspan="6">Project Name: Harbor and Outfall Monitoring — MWRA Contract No. S186</td></tr>
<tr><td colspan="3">Sampling Site:
Weather:</td><td colspan="3">Date:
Recorded by:</td></tr>
<tr><td colspan="3">Study____ Year____
Event____ Station____
Visit____ Sample____
Bottle____ Composite____
Comment________</td><td colspan="3">Study____ Year____
Event____ Station____
Visit____ Sample____
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Comment________</td><td colspan="3">Study____ Year____
Event____ Station____
Visit____ Sample____
Bottle____ Composite____
Comment________</td></tr>
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<tr><td colspan="2"></td><td colspan="2"></td><td colspan="2"></td></tr>
<tr><td colspan="2"></td><td colspan="2"></td><td colspan="2"></td></tr>
<tr><td colspan="6">PLEASE RETURN THE ORIGINAL OF THIS COMPLETED CHAIN-OF-CUSTODY FORM TO DAVID MITCHELL-ENSR</td></tr>
</table>

Figure 5a. Chain of Custody Form for Flounder Scales

<table>
<tr><td colspan="6">CHAIN-OF-CUSTODY RECORD</td></tr>
<tr><td colspan="6">For: Flounder Histology Samples</td></tr>
<tr><td colspan="6">Project Name: Harbor and Outfall Monitoring — MWRA Contract No. S186</td></tr>
<tr><td colspan="3">Sampling Site:
Weather:</td><td colspan="3">Date:
Recorded by:</td></tr>
<tr><td colspan="3">Study__________ Year____________
Event__________ Station__________
Visit__________ Sample___________
Bottle_________ Composite________
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Event__________ Station__________
Visit__________ Sample___________
Bottle_________ Composite________
Comment_________________________________</td></tr>
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Visit__________ Sample___________
Bottle_________ Composite________
Comment_________________________________</td></tr>
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<tr><td colspan="6">PLEASE RETURN THE ORIGINAL OF THIS COMPLETED CHAIN-OF-CUSTODY FORM TO DAVID MITCHELL-ENSR</td></tr>
</table>

Figure 5b. Chain of Custody Form for Flounder Histology Samples

CHAIN OF CUSTODY RECORD - INORGANIC CHEMISTRY ANALYSES

PROJECT NAME: MWRA Fish and Shellfish Task

Study_____	Year_____
Event_____	Station_____
Visit_____	Sample_____
Bottle_____	Composite_____
Comment_____	Cooler_____

BATCH NUMBER:
MATRIX:

Digestion Solution				
Relinquished By:	Date:	Received By:	Date:	Storage Location:

Figure 5c. Chain of Custody Form for Flounder/Lobster/Mussel Inorganic Chemistry Analyses

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CHAIN OF CUSTODY RECORD - ORGANIC CHEMISTRY ANALYSES

PROJECT NAME: MWRA Fish and Shellfish Task

Study_____	Year_____
Event_____	Station_____
Visit_____	Sample_____
Bottle_____	Composite_____
Comment_____	Cooler_____

BATCH NUMBER:
MATRIX:

GC/ECD Extract Transfer				
Relinquished By:	Date:	Received By:	Date:	Storage Location:

GC/MS Extract Transfer (edible tissue samples only)				
Relinquished By:	Date:	Received By:	Date:	Storage Location:

Figure 5d. Chain of Custody Form for Flounder/Lobster/Mussel Organic Chemistry Analyses

R:\PUBS\PROJECTS\4501005\035.TBL

CHAIN-OF-CUSTODY RECORD

For: Lobster Chemistry Samples

Project Name: Harbor and Outfall Monitoring — MWRA Contract No. S186

Sampling Site: Weather:	Date: Recorded by:
Study____ Year____ Event____ Station____ Visit____ Sample____ Bottle____ Composite____ Comment____	Study____ Year____ Event____ Station____ Visit____ Sample____ Bottle____ Composite____ Comment____
Study____ Year____ Event____ Station____ Visit____ Sample____ Bottle____ Composite____ Comment____	Study____ Year____ Event____ Station____ Visit____ Sample____ Bottle____ Composite____ Comment____
Study____ Year____ Event____ Station____ Visit____ Sample____ Bottle____ Composite____ Comment____	Study____ Year____ Event____ Station____ Visit____ Sample____ Bottle____ Composite____ Comment____
Study____ Year____ Event____ Station____ Visit____ Sample____ Bottle____ Composite____ Comment____	Study____ Year____ Event____ Station____ Visit____ Sample____ Bottle____ Composite____ Comment____

Released By/Date/Time/Company	Transporter/Airbill #	Received By/ Date/Time/Company

PLEASE RETURN THE ORIGINAL OF THIS COMPLETED CHAIN-OF-CUSTODY FORM TO DAVID MITCHELL-ENSR

Figure 5e. Chain of Custody Form for Lobster Chemistry Samples

CHAIN-OF-CUSTODY RECORD	
For: Mussel Chemistry Samples	
Project Name: Harbor and Outfall Monitoring — MWRA Contract No. S186	
Sampling Site: Weather:	Date: Recorded by:
Study____ Year____ Event____ Station____ Array____ Cage #____ Comment____ Cooler____	Study____ Year____ Event____ Station____ Array____ Cage #____ Comment____ Cooler____
Study____ Year____ Event____ Station____ Array____ Cage #____ Comment____ Cooler____	Study____ Year____ Event____ Station____ Array____ Cage #____ Comment____ Cooler____
Study____ Year____ Event____ Station____ Array____ Cage #____ Comment____ Cooler____	Study____ Year____ Event____ Station____ Array____ Cage #____ Comment____ Cooler____
Study____ Year____ Event____ Station____ Array____ Cage #____ Comment____ Cooler____	Study____ Year____ Event____ Station____ Array____ Cage #____ Comment____ Cooler____

Released By/Date/Time/Company	Transporter/Airbill #	Received By/ Date/Time/Company

PLEASE RETURN THE ORIGINAL OF THIS COMPLETED CHAIN-OF-CUSTODY FORM TO DAVID MITCHELL-ENSR

Figure 5f. Chain of Custody Form for Mussel Chemistry Samples

Upon completion of the sampling, custody of samples will be transferred to the appropriate laboratory. Laboratory custody of all samples will be the responsibility of the subcontractor. Upon receipt of samples at the laboratory, the recipient will examine the samples received, verify that the information recorded on the copy of the chain-of-custody forms is accurate, and log the samples into the laboratory by signing the chain-of-custody form on the *Received By* line, and by entering the date and time of sample receipt. Any inconsistencies between samples listed as having been released and samples that were actually received, or any damage to containers, labels, etc., will be noted in the laboratory sample log book and immediately communicated to the ENSR Task Manager. Sample numbers that include the complete field ID number will be used to track the samples through the laboratory. All archived samples will remain in the custody of the appropriate subcontractor laboratory for a period of 1 year after sample collection, at which time the MWRA will be contacted about their disposition. All data generated for this study will be maintained for 6 years, after which time MWRA will be contacted.

13.3 Histology Samples

The laboratory custodian of samples for histologic analyses will be Dr. Michael Moore, of WHOI. He will personally deliver the samples to be histologically processed to the Laboratory for Marine Animal Health at the Marine Biological Laboratory in Woods Hole where chain-of-custody forms will be signed by the receiving histology technician Michelle McCafferty. The tissue slices will be embedded in the same tissue cassettes labelled at the time of collection. Sample numbers will be copied from the cassettes to the slides at the time of sectioning the embedded blocks. The blocks and slides will be returned to Moore in person, chain-of-custody forms signed again and all histology material thereafter will be archived at WHOI.

13.4 Samples for Tissue Chemistry

The laboratory custodian of samples for organic analyses will be Mr. John Brown of ADL. The laboratory custodian of samples for inorganic (metal) analyses will be Mr. Ravi Ika of Envitec. They will be responsible for receiving samples (by signing the chain-of-custody) for tissue chemical analysis. Unique laboratory sample identification numbers will be used to track samples through the chemistry laboratory. The root of these numbers will be the unique sample ID assigned to each sample in the field. A suffix will be added to each root to act as a descriptor of the chemical analyses. For example, the suffix "-ORG" may be added to signify the organics aliquot of a flounder tissue sample.

14.0 CALIBRATION PROCEDURES AND PREVENTIVE MAINTENANCE

Logs of maintenance, calibrations, and any repairs made to instruments will be maintained by the respective subcontractors. Maintenance of and repairs to instruments will be in accordance with manufacturers' manuals and facility SOPs.

14.1 Navigation Equipment

Once the 12 VDC power supply for the Northstar 941XD navigation system has been switched on, there is typically no other setup interaction necessary between the Seasoft operator and the navigation system. The GPS will conduct an automatic self-test, and then begin acquiring satellites and a beacon. This process normally takes 2 to 5 minutes. An error message will be displayed if the system has trouble acquiring satellites or a beacon. For each survey, the GPS position will be verified by comparing it to previously located benchmarks. At a minimum, the position will be verified once, at the dock. In addition, the geometry and number of satellites will be checked periodically throughout the survey. Cable connections and antenna mounts and brackets will be inspected and secured at regular intervals.

14.2 Field Equipment

Instruments will be calibrated according to the following methods:

- Measuring boards used to measure flounder will be wiped dry after measuring every 10th specimen and will be rinsed after sampling has been completed at each sampling site.
- The Chatillon fish scale, Model No. 70-2030, will be dried after weighing every 10th fish and will be calibrated with a known weight after sampling has been completed at each sampling site. The scale will be inspected prior to measuring each fish to ensure that it reads zero.
- The knife used to remove scales from flounder will be wiped clean after collecting samples from each specimen.
- Calipers used to measure the carapace length of specimens will be wiped dry after sampling has been completed at each sampling site.

14.3 Laboratory Equipment

The primary analytical instrument used for the histological analyses is a Zeiss photomicroscope. No calibrations are required. When not in use, the microscope will be covered to prevent excess dust from settling on it. The lenses will be cleaned periodically with lens cleaning fluid.

14.3.1 Instrumentation for Organic Chemical Analysis

Analytical instruments will be calibrated before sample analysis. Response factors (RF) will be generated for each target analyte using the following equation:

$$RF = \frac{A_x}{A_{IS}} \times \frac{C_{IS}}{C_x}$$

where:
- A_x = peak area of the analyte in the calibration standard
- A_{is} = peak area of the appropriate internal standard in the calibration standard
- C_x = concentration of the analyte in the calibration standard
- C_{is} = concentration of the appropriate internal standard in the calibration standard.

Five concentrations of standard solutions that encompass the expected range in sample concentrations will be analyzed. Initial calibrations will be acceptable if the relative standard deviations (RSD) are ≤30% of the mean for each individual analyte, and the mean of all analyte RSDs is ≤15%.

The system calibration will be verified a minimum of once every 24 hours using a mid-range calibration check. Using the mean RF of each analyte from the initial calibration, the percent difference between those mean values and the RFs from the midrange calibration checks will be calculated. The percent difference is calculated by:

$$\% \text{ Difference} = [(RF_i - RF_r) / RF_i] \times 100$$

where
- RF_i = average response factor from the initial calibration, and
- RF_r = response factor from the midrange calibration check.

The calibration checks will be acceptable under the same criteria as the initial calibration (i.e., 30% for individual analytes, 15% for the means). If the percent difference between the RFs is greater than the acceptability criteria, remedial maintenance will be performed on the instrument, a new initial calibration will be performed, and the affected batch of samples may be reanalyzed. Because GC/ECD and GC/MS analyses are multicomponent analyses, it may not be necessary to reanalyze all samples. For example, if only certain analytes are detected in a sample, and the calibration is acceptable for those particular analytes, the sample should not require reanalysis. The decision as to whether reanalysis is necessary will be made by the laboratory task manager, with approval by the ENSR Task Manager. Deviations from calibration or data objectives will be documented in the project files and reported to the MWRA.

Samples analyzed by GC/ECD and GC/MS will be bracketed by two acceptable calibrations, initial or check. Analytes will be quantified by using the average RFs for that individual analyte generated from the initial calibration unless otherwise stated.

14.3.2 Instrumentation for Metal Analysis

Calibration standards will be prepared each day and tissue digestion solutions will be quantified by ICP-MS for all metals (except mercury) using external standards. Matrix interferences will be corrected using an on-line internal standard. A calibration correlation coefficient value of 0.997 or better will be achieved before proceeding for sample analysis. Calibration accuracy will be checked with an SRM (NIST 1643c Metals in Water). NIST 1643c sample will be analyzed for every 10 samples to ensure continued accuracy. Measurements that are not bracketed by an accuracy check standard within 10% of its true value will be rejected and reanalyzed after corrective action is taken (as needed). ICP-MS measurements will be made in triplicate for each sample; if the RSD of replicate measurements is greater than 5%, then the sample measurement will be rejected unless the ion counts are very low and small differences result in high RSD values.

The CVAAS measurements of mercury will be quantified by standard comparisons; mercury calibration standards will be prepared the day of analysis, and samples will be quantified within the linear range of the instrument and below the highest calibration standard. Instrument performance will be monitored using continuing accuracy check standards (with a 10% acceptance criteria), prepared by an analyst other than the analyst that prepares the calibration standards. Samples will be analyzed once for quantitation; all duplication exercises will be laboratory or field duplicates. Sample quantitations will proceed only if the calibration standard curve is linear with a correlation coefficient of 0.99 or better.

If the target correlation coefficient is not obtained for the ICP-MS, then the instrument operation and integrity will be assessed and analytical standards evaluated. Mass calibration on the instrument will be performed periodically to correct drifts. Necessary remedial action will be taken, and the calibration procedure repeated until a satisfactory calibration for each trace metal can be obtained. Any sample concentrations that are above the highest calibration standard will be reanalyzed with appropriate dilutions.

14.4 Instrument Maintenance

Analytical instrumentation will be properly calibrated and maintained in accordance with laboratory SOPs, manufacturers' instructions, and analytical methods. A log will be kept for each analytical instrument and will contain a record of all routine maintenance and repairs. Procedures for maintenance of the more complex analytical equipment are described below.

14.4.1 Gas Chromatograph Mass Spectrometer

Detector response (electron-capture detectors and mass spectrometer) and capillary column performance will be monitored/calibrated daily by injection of GC standards containing known amounts of targeted compounds (e.g., PAH mixture, pesticides, and PCB mixtures). Both the responses per unit amount and the resolution of specific components will be monitored. If any evidence of chromatographic column performance deterioration is observed, the column will be replaced.

14.4.2 ICP-MS

Maintenance of the ICP-MS instrumentation will include complete cleaning of sample and skimmer cones, replacing sampling tubes, and optimizing the instrument sensitivity by adjusting and cleaning the lenses. The base vacuum, operating vacuum, and gas flow rates will also be checked and adjusted as necessary.

15.0 DOCUMENTATION, DATA REDUCTION, AND REPORTING

15.1 Documentation

Documentation will include sample collection logs, chain-of-custody forms, and laboratory records. Sample collection information will be recorded on standard forms that, at a minimum, should include sample location, time and date, sampler's identification, and sample ID number. Examples of sample collection logs are given in Section 12.0. Chain-of-custody records are discussed in Section 13.0.

Initially, all laboratory data will be recorded either (1) electronically onto computer storage media from laboratory data systems or (2) manually into laboratory notebooks or on established data forms. All notes will be written in ink. Corrections to hand-entered data will be initialed, dated, and justified. Completed forms, laboratory notebooks, or other forms of hand-entered data will be signed and dated by the individual entering the data. It will be the responsibility of the laboratory managers to ensure that all data entries and hand calculations are verified. Laboratory records of sample preparation will be maintained.

Data loading documentation will include diskettes received from subcontractor laboratories, spreadsheets, and Microsoft Access files used for loading, as well as results of checks made during loading and scripts or Access files fully documenting any corrections made during loading.

15.2 Data Reduction

Data reduction involves the process of converting raw numbers into data that have direct physical, biological or chemical meaning and can be compared statistically.

15.2.1 Navigation Data

The vessel otter-trawl tracks, lobster-pot deployment, and mussel array deployment positions will be reported as latitude/longitude and will be plotted on map form. The plots will be stored in project files to allow inspection by QA staff.

15.2.2 Histology Data

The severity of each lesion will be rated on a scale of 0 to 4, where: 0 = absent; 1 = minor; 2 = moderate; 3 = severe; and 4 = extreme. For each lesion and each fish, a histopathological index will then be calculated as a mean of scores from three slides. Data resulting from the assignment of scores to the various lesions will be transferred into Oracle for generation of final report tables. Analyses of variance will be used to compare lesions from site to site and from 1994 to 1995.

15.2.3 Tissue Chemistry Data

GC/MS data will be acquired and reduced on Hewlett-Packard A-series software. GC/ECD data will be acquired and reduced on a VG Minichrome Data Chromatography software. Organic chemistry data will be reported in units of ng/g dry-weight, based on the surrogate internal standards.

Statistical evaluations will be performed on all QC samples. Percent recoveries of the spiked analytes will be calculated for all matrix spike and matrix spike duplicate samples, SRMs, and surrogates as follows:

$$\%Recovery = \frac{Amount\ Detected(ng)}{Amount\ Expected(ng)} \times 100$$

Additionally, RPD between the MS and MSD samples will be calculated as follows:

$$RPD = \frac{2 \times (A_{MS} - A_{MSD})}{A_{MS} + A_{MSD}} \times 100$$

where A_{MS} = amount of analyte detected in MS sample
A_{MSD} = amount of analyte detected in MSD sample.

The RPD between sample duplicates will be calculated as follows:

$$RPD = \frac{2 \times (C_1 - C_2)}{(C_1 + C_2)} \times 100$$

where C_1 = concentration (ng/g) of analyte detected in sample 1
C_2 = concentration (ng/g) of analyte detected in sample 2

Data quality objectives for these calculations are presented in Table 10.

15.3 Reporting

Three formats will be used to report the fish and shellfish monitoring data to MWRA

(1) Data submitted for inclusion in the Harbor Studies Database
(2) Data presented in annual data reports

(3) Data summarized and interpreted in annual synthesis reports.

15.3.1 Harbor Studies Database

Only data that have been designated as final by the Project Area Manager will be loaded into ENSR's copy of the Harbor Studies Database. All data will be loaded into the database by ENSR data management staff following the formats described below. Upon receipt, each diskette will be logged in and assigned an unique log in identifier. Any changes or additions to data, necessary for loading into the database, will be made using well-documented scripts that indicate the original values. The original diskette, scripts, and data-loading documentation will be filed at ENSR according to the log-in identifier. The data sources notebook will contain the chain-of-custody forms and data entry information.

Sample Collection Data

Sample collection data contained in the sample chain-of-custody form will be included in the sample Oracle table. Columns will include sample_id, time/date_id, bottle_id, composite_id, and cooler_id.

Analytical and Experimental Data

All data generated by ENSR subcontractors will be either electronically transferred from the instrument to Oracle tables or a PC-based spreadsheet, or read from the instrument display (or optical field or a microscope) and manually entered into laboratory notebooks or data sheets. Data in laboratory notebooks or data sheets will be manually entered into a PC-based spreadsheet or into Oracle through a Microsoft Access interface. Subcontractor spreadsheet data will be loaded into Oracle and checked by ENSR data management staff. Columns include sample_id, study_id, bottle_id, fraction, param_code, value, val_qual, unit_code, anal_ref, anal_date, meth_code, detect_limit, instr_code, QC_code, lab_sample_id, and _lab_id, batch_no, and comments.

15.3.2 Data Reports

Data reports will be submitted to MWRA in both hard-copy and electronic forms. Data will be presented in tables containing the results of individual sample analysis plus QC data.

16.0 DATA VALIDATION

All data reported for this project will be reviewed to check for errors in transcription, calculation, or computer input by the technical staff of the appropriate laboratory. The validation procedures that will be performed are:

- 100% of data that are hand-entered into a database or spreadsheet will be verified for accuracy either by (1) printing the spreadsheet and proofreading against the original hand entry or by (2) duplicate entry into the database and comparison of the entries to detect any differences. These tasks will be carried out by two people and documented for each data set.

- All manual calculations will be checked for accuracy by a second staff member.

- Calculations performed by software will be checked at a frequency sufficient to ensure the accuracy of the calculations. All data-reduction algorithms will be verified prior to final data submission.

- Subsets of the analytical data will be reviewed by in-house or subcontractor data validators. The data will be reviewed for adherence to analytical protocols and to pre-established criteria (e.g., for holding times, surrogate recoveries, initial and continuing calibration, matrix spikes, laboratory duplicates, blank contamination, SRM recoveries).

- Database staff will check the received data and associated documentation for completeness, freedom from errors, and technical reasonableness.

- All new software developed for this task will be validated before entry of data.

The ENSR Project Area Manager will be responsible for validation of all data generated by ENSR to ensure that the data are accurate, complete, and scientifically reasonable. Subcontractors will be responsible for conducting similar data validations. As an additional data validation step, the ENSR Project Area Manager or his designate will review all subcontractor data for technical reasonableness.

17.0 PERFORMANCE AND SYSTEMS AUDITS

This project will be monitored by the ENSR Project QA Director. All tabular and graphic data reported in deliverables and associated raw data generated by ENSR will be reviewed by the Project QA Director or his/her designee. Raw data will be reviewed for traceability, accuracy, completeness, and proper documentation.

All deliverables generated during the course of this project will be submitted to an internal review prior to delivery of drafts to MWRA.

Audits of the subcontractor laboratory data-collection programs will be the responsibility of the subcontractor. During the time work is in progress, an inspection will be conducted by the Subcontractor QA Officer or their designee to evaluate the laboratory data-production process. All data must be reviewed by the Subcontractor QA Officer prior to submission to the ENSR Project Area Manager and must be accompanied by a signed QA statement that describes the types of audits and reviews conducted and any outstanding issues that could affect data quality.

Performance audits, procedures used to determine quantitatively the accuracy of the total measurement system or its components, will be the responsibility of subcontractor laboratories and may include internal performance evaluation samples and participation in external certification programs.

18.0 CORRECTIVE ACTION

Identification of problems regarding technical performance is the responsibility of all staff members working on this project. Responsibility for overall conduct of the project, including schedule, costs, and technical performance lies with the ENSR Project Manager. The Project Manager is responsible for identifying and resolving problems that (1) have not been addressed promptly or successfully at a lower level, (2) influence other components of the project, (3) require changes in this CW/QAPP, or (4) require consultation with ENSR management or with MWRA.

Technical problems relating to sample collection (schedule changes, modifications to the sampling plan, etc.) will be resolved through discussion with the MWRA Task Manager and ENSR Project Area Manager. Problems relating to the overall successful completion of the project will be reported to the MWRA Task Manager in a timely manner for discussion and resolution between the ENSR and MWRA managers.

Identification of problems and corrective action at the laboratory level will be resolved by the laboratory staff. Issues that affect schedule, cost, technical performance, or data quality will be reported to the ENSR Task Manager or the ENSR Project Area Manager. They will be responsible for evaluating the overall impact to the project and for discussing corrective actions with the MWRA Task Manager.

A QA/QC Corrective Action Log will be maintained by the ENSR Project QA Director and submitted to MWRA Task Manager at quarterly intervals. Figure 6 presents a sample corrective action log. The log will include documentation of QA/QC activities as they occur, descriptions of the methods and procedures recommended to prevent the problem from reoccurring, and verification that these actions have corrected the problem.

19.0 REPORTS

Reports that will be generated under Tasks 21 - 25 include survey plans and survey reports for each of the three surveys conducted under Tasks 21-23 and data reports (described below). Each survey plan will follow the new guidelines established by EPA for use of the OSV *Anderson.* Three copies of the final survey plan will be submitted to MWRA at least two weeks prior to the survey. No draft survey plans will be prepared. Survey reports will be submitted to MWRA within two weeks after each survey demobilization.

19.1 Tissue Chemistry Data Reports (Task 24)

The data report will be a tabular summary of results and a brief discussion of any deviations from this CW/QAPP.

Data from chemical analyses of tissues will also be used in reports to be prepared under Task 33, specifically the fish and shellfish monitoring annual synthesis report (Task 33.b) and the annual toxics review (Task 33.8).

19.2 Histology Data Reports (Task 25)

Histological data reports (Task 25) will be a tabular summary of results and a brief discussion of any deviations from this CW/QAPP. The histopathology will be discussed under the annual fish and shellfish monitoring synthesis report (Task 33.b), and will include the following:

ENSR

Corrective Action Log

Program Title: MWRA Harbor and Outfall Monitoring Program

MWRA Task Description:_____ MWRA Task Number:_____

Project Area Leader:_____ Task Leader:_____

Date of Occurrence	Name and Initials of Recorder	Description of Problem	Description of Corrective Action	Approval (Task Leader) and Date

Figure 6. Corrective Action Log

SUMMARY
CONCLUSIONS
RECOMMENDATIONS
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METHODS
- Stations and Sampling
- Dissection of Fish
- Age Determination
- Histological Processing
- Histological Analysis
- Data Analysis
- QA/QC

RESULTS AND DISCUSSION
- Fish Collected
- Age/Length Parameters
- Interstation Comparison of Lesion Prevalence
- Interstation Comparison of Histopathological Indices
- Age Effects on Lesion Prevalence

REFERENCES

19.3 Mussel Biological Condition Data Reports (Task 25)

Mussel Biological Condition Data Reports (Task 25) will be a tabular summary of results and a brief discussion of any deviations from this CW/QAPP. The mussel condition data will be discussed under the annual fish and shellfish monitoring synthesis report (Task 33.b), and will include the following:

SUMMARY
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RECOMMENDATIONS
INTRODUCTION
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- Mussel Collection
- Mussel Deployment
- Mussel Retrieval
- Biological Analyses
- Statistical Analysis

RESULTS AND DISCUSSION
- 1995 Biological Data

Survival
Sexual Maturity
Growth and Condition
Temporal Trends
REFERENCES

20.0 REFERENCES

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