COMBINED WORK/QUALITY ASSURANCE PROJECT PLAN (CW/QAPP)

for

FISH AND SHELLFISH MONITORING: 1993-94

Tasks 14, 15, 16, and 17
MWRA Harbor and Outfall Monitoring Project

submitted to

MASSACHUSETTS WATER RESOURCES AUTHORITY
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MWRA Harbor and Outfall Monitoring Project — Fish and Shellfish Monitoring

2. PROJECT REQUESTED BY

Massachusetts Water Resources Authority

3. DATE OF REQUEST

December 6, 1992

4. DATE OF PROJECT INITIATION

December 6, 1992

5. PROJECT MANAGEMENT

Dr. Michael Connor, MWRA Director of Environmental Quality Department
Dr. Michael Mickelson, MWRA Harbor and Outfall Monitoring Project Manager
Mr. Ken Keay, MWRA Project Area Manager

Dr. Carlton Hunt, Battelle Project Manager for Harbor and Outfall Monitoring
Dr. Robert Hillman, Battelle Fish and Shellfish Project Area Leader
Dr. Carlton Hunt, Battelle Acting Technical Director

6. QUALITY ASSURANCE (QA) MANAGEMENT

Ms. Rosanna Buhl, Battelle Project QA Officer

7. PROJECT DESCRIPTION

7.1 Background

The Massachusetts Water Resources Authority (MWRA) is implementing Phase I of a long-term monitoring plan (MWRA, 1991) for the MWRA effluent outfall that will be located in Massachusetts Bay (see Figure 1). The goal of Phase I monitoring 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 discharge permit.

To help determine the body burden of toxic substances and to assess the health of winter flounder *(Pleuronectes americanus)* and lobster *(Homarus americanus),* one survey will be conducted in Boston Harbor, Massachusetts Bay, and Cape Cod Bay (hereafter: Boston Harbor and the Bays) during each of 1993 and 1994 to collect specimens for analysis. This Combined Work/Quality Assurance Project Plan (CW/QAPP) describes the sampling and analysis activities associated with the flounder and lobster surveys that will be conducted under Tasks 14 and 15, and the histological and chemical analyses that will be conducted under Tasks 16 and 17 of the MWRA Contract S138. Tasks 14 and 16 are years 3 and 4 of an ongoing project in the Bays (see Moore and Stegeman, 1993) and builds on several years of monitoring in Boston Harbor. Data quality requirements and assessments, project management (organization and responsibilities of Battelle staff and subcontractors), and a schedule of activities and deliverables associated with the fish and shellfish monitoring surveys are also described in this CW/QAPP.

This CW/QAPP conforms to the format used by the U.S. Environmental Protection Agency (EPA), Office of Water, and it provides the information necessary to implement the monitoring described in the Outfall Monitoring Plan (MWRA, 1991). Separate survey plans will be developed for each survey. The survey plans will provide the operational details required to conduct each survey, and will describe participating staff, detailed schedule, and specific equipment.

7.2 Objective and Scope

The overall objective of the fish and shellfish monitoring is to define the baseline of winter flounder and lobster 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 and lobster). With a sound baseline characterization of the health of

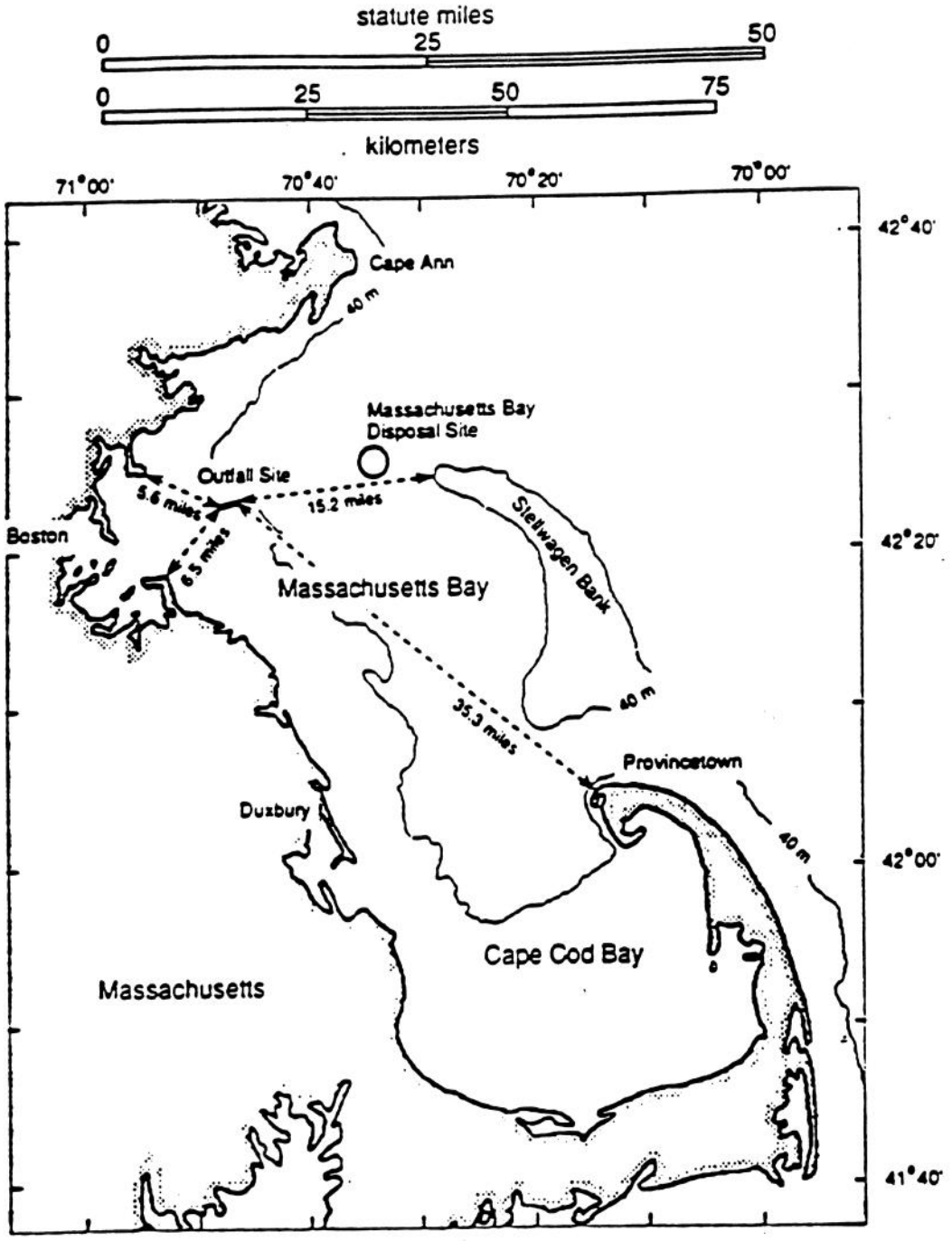

Figure 1. Boston Harbor and the Bays.

winter flounder and lobster 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 four tasks included in this CW/QAPP are described below.

7.2.1 Flounder Survey (Task 14)

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, and chemical analyses of tissue to determine baseline health and tissue burden of contaminants. Specimens will be collected during surveys conducted in April 1993 and 1994.

7.2.2 Lobster Survey (Task 15)

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 April 1993 and 1994.

7.2.3 Histological Analysis (Task 16)

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 14. 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.

7.2.4 Tissue Chemical Analyses (Task 17)

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 and lobster collected under Tasks 14 and 15.

7.3 Data Usage

7.3.1 Histological Analysis (Task 16)

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.

7.3.2 Tissue Chemical Analyses (Task 17)

Chemistry data will be used to determine the concentrations of organic and inorganic (metals) contaminants in flounder liver, lobster hepatopancreas, and flounder and lobster edible tissue (filet; claw and tail 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.

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.

7.4 Technical Approach

7.4.1 Flounder/Lobster Surveys (Tasks 14 and 15)

A three-day survey will be conducted during April 1993 and April 1994. The flounder and lobster surveys will be conducted concurrently. The general technical approach to conducting each survey includes the following components:

- Preparation of survey plans for each survey
- Collection of winter flounder and lobster
- Preparation of survey report for each survey

A survey-specific plan will be prepared for each of the two surveys. This plan will include specific activities for collection of both winter flounder and lobster. These plans will be delivered to MWRA no later than one week before the start of each survey. These survey plans will follow the guidelines established by the EPA for use of the OSV *Anderson* and will include the following information:

- Schedule of operations
- Background information
- Survey location and description
- Survey/sampling methodologies
- Navigation and positioning control

Battelle will conduct two flounder/lobster surveys. Five sites will be sampled to collect winter flounder for histological and chemical analyses. Three sites will be sampled to collect lobster for chemical analyses. Table 1 provides the sampling sites and locations. Figure 2 illustrates the sampling locations in Boston Harbor and the Bays.

Table 1. Sampling Sites and Locations for Flounder/Lobster Survey.

	Location	
Sampling Site	***Latitude***	***Longitude***
Deer Island Flats (Boston Harbor)[a]	42°20.4′	70°58.4′
Off Nantasket Beach	42°17.6′	70°52.2′
Broad Sound	42°24.4′	70°57.2′
The site of the future effluent outfall[a]	42°23.1′	70°49.3′
Eastern Cape Cod[a]	42°56.2′	70°06.6′

[a] Sampling site for lobsters

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. The gonads of each flounder will be examined to determine sexual maturity. A minimum of five female and five male specimens will be collected (e.g., 5 males, 45 females; 5 females, 45 males). All specimens will be weighed, and standard and fork length will be determined. Scales will be taken from each specimen. For specimens greater than 35 cm, otoliths will also be collected only if they are needed to verify ages determined from the fish scales. 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.

A string of five lobster pots (collectively referred to as a lobster trawl) will be set to collect 10 commercially harvestable lobsters at each site. 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 two weeks after each survey, a survey report will be prepared. The results of the winter flounder and lobster surveys will be provided in one survey report. Each report will contain the following information:

- Winter flounder collection operations
- Lobster collection operations
- Maps of actual survey tracks for each day of each survey
- Problems experienced, and recommended response

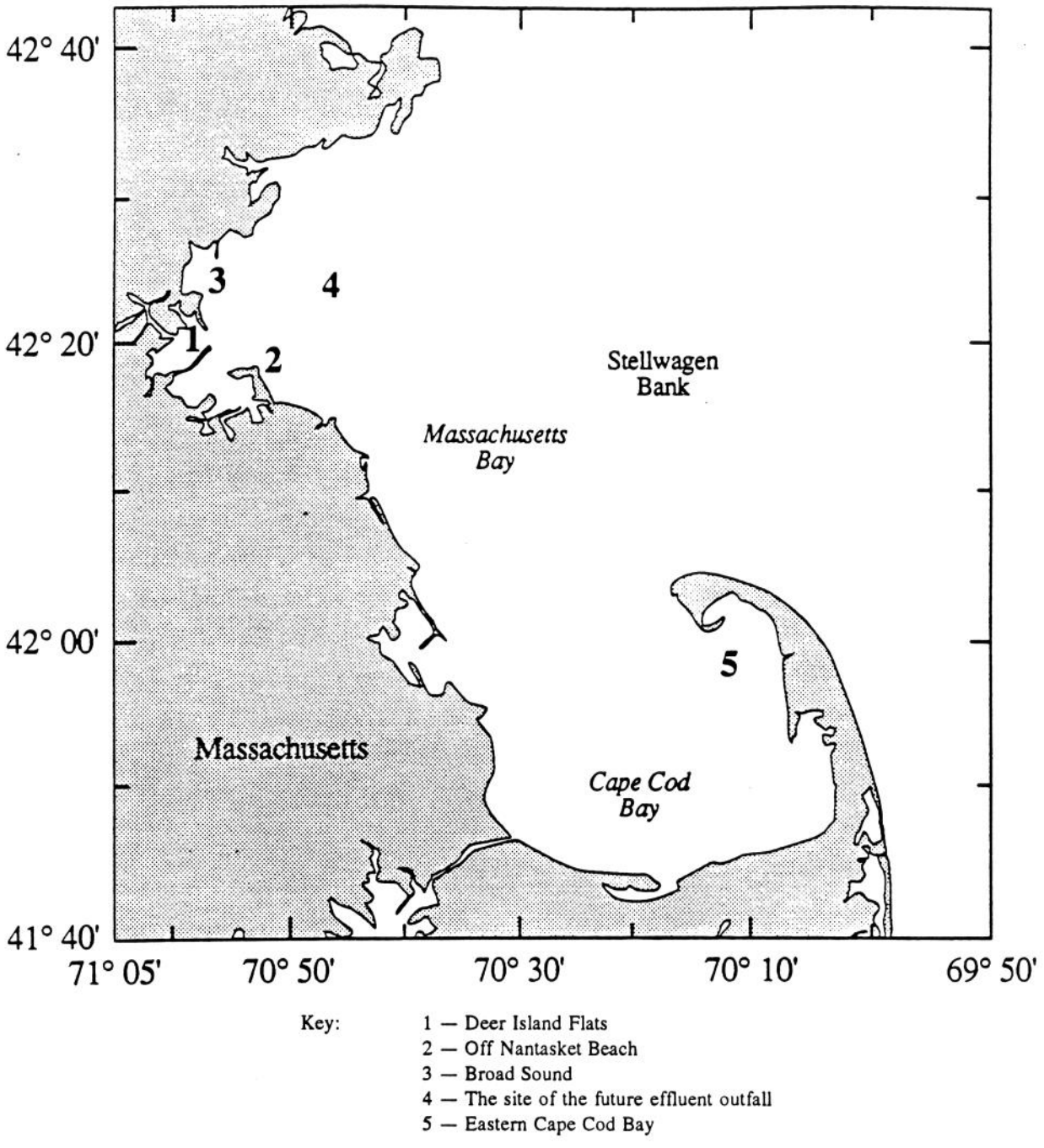

Figure 2. Location of Sampling Sites for Flounder and Lobster Surveys.

7.4.2 Histological Analyses (Task 16)

Fifty flounder will be selected from each of the five sampling sites for the suite of histological analyses. Two sections, 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 1500 slides will be prepared and examined each year (1993 and 1994). 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 or otolith, if necessary, from that specimen.

7.4.3 Tissue Chemical Analyses (Task 17)

Three pools of samples will be created from each of the 10 flounder and 10 lobster samples collected at each sampling site. Forty-eight (48) samples from each survey will be analyzed for tissue chemical analyses:

- Flounder — 3 pools × 5 sampling sites × 2 sample types (edible tissue, liver) = 30 samples
- Lobster — 3 pools × 3 sampling sites × 2 sample types (edible tissue, hepatopancreas) = 18 samples

A total of 96 tissue samples from both surveys will be analyzed for lipid content, metals, and organic analytes, including polynuclear aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), and chlorinated pesticides.

7.5 Monitoring Parameters and Collection Frequency

Table 2 summarizes the number of organisms and the types of analyses that will be conducted on samples collected from each station. Table 3 summarizes the parameters that will be measured in Task 16. Table 4 summarizes the primary chemical parameters that will be measured for each organisms or sample type (Task 17). Table 5 lists the specific analytes that will be measured.

8. PROJECT FISCAL INFORMATION

This project is being carried out under the Harbor and Outfall Monitoring contract (Contract No. S138) between MWRA and Battelle Ocean Sciences.

Table 2. Field Samples and Measurements

Organism	*Parameter*	*Number*	*Container*	*Shipboard or Laboratory Processing/ Preservation*
Winter flounder	Chemistry - liver	10	Clean, labeled jar	Laboratory: Freeze, if not processed immediately
	- edible tissue	10		
	Histology	50	Clean, labeled jar	Laboratory, Shipboard: 10% neutral buffered formalin
	Age (scales/ otoliths)	50	Age envelope	Shipboard: Clean mucous from sampling area of fish before taking scales/ clean tissue from otoliths
	Visual	50	N/A	Shipboard: Describe qualitatively
Lobster	Chemistry - hepatopancreas	10	Clean, labeled jar	Laboratory: Freeze, if not processed immediately
	- edible tissue	10		
	Visual	10	N/A	Shipboard: Describe qualitatively

Table 3. Histology Analysis Parameters.

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

Table 4. Chemistry Analysis Parameters.

Organism	*Number/Type of Samples*	*Parameters*
Flounder	30/filet	Mercury PCB Chlorinated pesticides Lipids
Flounder	30/liver	Trace metals PCB PAH Chlorinated pesticides Lipids
Lobster	18/meat	Mercury PCB Chlorinated pesticides Lipids
Lobster	18/hepatopancreas	Trace metals PCB PAH Chlorinated pesticides Lipids

Table 5. Analytes Included in Tissue Chemistry Analyses.

Major Metals
- Al Aluminum
- Fe Iron

Trace Metals[a]
- Ag Silver
- Cd Cadmium
- Cr Chromium
- Cu Copper
- Hg Mercury[b]
- Ni Nickel
- Pb Lead
- Zn Zinc

Polychlorinated biphenyls (PCBs)[c]
- 2,4,-Cl_2(8)
- 2,2′,5-Cl_3(18)
- 2,4,4′-Cl_3(18)
- 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]
- naphthalene
- C_1-naphthalenes
- C_2-naphthalenes
- C_3-naphthalenes
- acenaphthylene
- acenaphthene
- C_1-fluorenes

Polynuclear Aromatic Hydrocarbons (PAHs) (continued)
- C_2-fluorenes
- C_3-fluorenes
- phenanthrene
- C_1-Phenanthrenes/anthracene
- C_2-Phenanthrenes/anthracene
- C_3-Phenanthrenes/anthracene
- C_4-Phenanthrenes/anthracene
- 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

Pesticides[c]
- 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

Lipids[c]

[a] Flounder liver; lobster hepatopancreas
[b] Flounder and lobster edible tissue
[c] Flounder edible tissue and liver; lobster edible tissue and hepatopancreas

9. SCHEDULE OF ACTIVITIES AND DELIVERABLES

The schedule of activities and deliverables for this project is tied to survey activities. Table 6 provides the 1993 and 1994 planned schedule for all survey plans, survey reports, and data reports required for Tasks 14, 15, 16, and 17.

The deliverables for Tasks 14, 15, 16, and 17 are (1) survey plans and survey reports for each of the two 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.

Table 6. Schedule of Deliverables.

Task	*Deliverable*	*Due Date*
Flounder/Lobster Surveys (Tasks 14 and 15)	1993 Survey Plan	March 31, 1993
	1993 Draft Survey Report	May 2, 1993
	1993 Final Survey Report	May 31, 1993
	1994 Survey Plan	March 31, 1994
	1994 Draft Survey Report	May 2, 1994
	1994 Final Survey Report	May 31, 1994
Histological Analysis (Task 16)	1993 Draft Data Report	Aug. 31, 1993
	1993 Final Data Report	Sept. 29, 1993
	1994 Draft Data Report	Aug. 31, 1994
	1994 Final Data Report	Sept. 29, 1994
Tissue Chemical Analysis (Task 17)	1993 Draft Data Report 1993 Final Data Report	Aug. 31, 1993 Sept. 29, 1993
	1994 Draft Data Report 1994 Final Data Report	Aug. 31, 1994 Sept. 29, 1994

10. PROJECT ORGANIZATION AND RESPONSIBILITIES

The project organization is shown in Figure 3. Dr. Michael Mickelson is the MWRA Project Manager. Mr. Ken Keay is the MWRA Project Area Manager. They will be informed of all matters pertaining to work described in this CW/QAPP. Dr. Carlton Hunt is the Battelle Project Manager responsible for the overall performance of this project. Dr. Robert Hillman is the Battelle Project Area Leader responsible for the conduct of the fish and shellfish monitoring tasks described in this CW/QAPP. Dr. Hillman, with the assistance of Mr. Carl

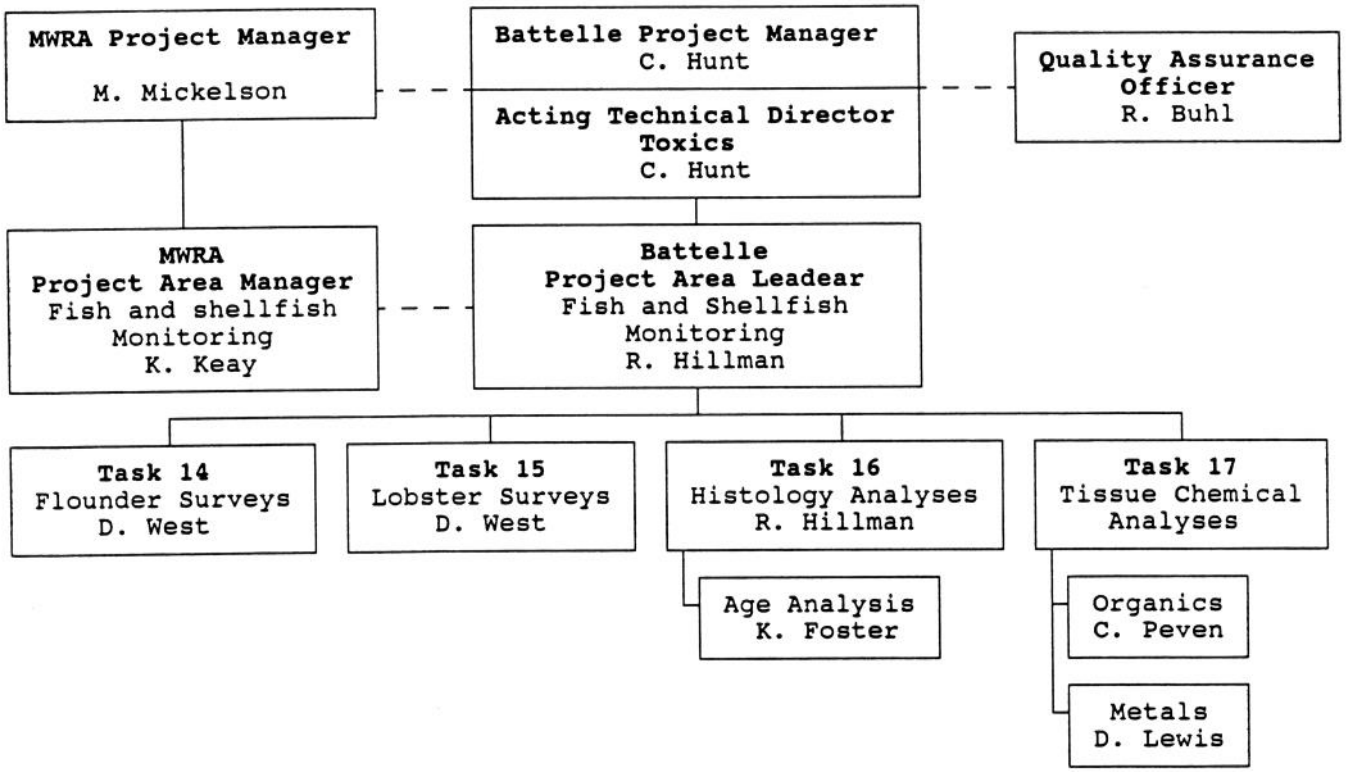

Figure 3. Fish and Shellfish Monitoring Project Organization.

Albro, will be responsible for coordinating all survey operations and for ensuring successful completion of each survey. It is expected that Ms. Deborah West will be the Chief Scientist on the surveys. The Chief Scientist for each survey will be responsible for preparing the survey plans and reports, and will oversee/direct survey activities. She will be assisted by Ms. Joanne Lahey during field activities.

Robert Hillman will oversee histological analyses of winter flounder liver samples (Task 16). Ms. Karen Foster, Age Analysis Task Leader, will oversee the fish scale readings and, if necessary, the otolith readings (Task 16). Ms. Carole Peven, Organic Chemistry Task Leader, will oversee chemical analyses for organic contaminants in the liver, hepatopancreas (lobster), and edible tissue (Task 17). Mr. Dion Lewis, Metal Chemistry Task Leader, will oversee chemical analyses for metals in the liver, hepatopancreas (lobster), and edible tissue (Task 17).

Ms. Rosanna Buhl, Project QA Officer, will administer the QA program for all technical activities conducted by Battelle.

11. DATA QUALITY REQUIREMENTS AND ASSESSMENTS

To ensure that all data generated during the conduct of Tasks 14, 15, 16, and 17 are of the highest 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

The Battelle Ocean Sampling System (BOSS) Navigation Subsystem will be used for positioning during all of the surveys. This system consists of a Northstar

Model-800 global positioning system (GPS) and LORAN-C Model-8000 system interfaced to a computer. Battelle proprietary software reads the latitude and longitude output of the Northstar system and displays this position on a color monitor that shows vessel position relative to station position.

11.1.1 Accuracy

All sampling will be conducted within the geographical boundaries of the sampling site. The geographical location of each sampling site will be stored in the BOSS system prior to conducting sampling. During high winds or in the presence of strong water currents, the vessel will be positioned 300 m upstream of station position at the start of a otter-trawl tow or deployment of lobster pots. The Northstar system has an absolute accuracy of 15-100 m, depending upon the implementation of Selective Availability (SA), which is adequate for the outfall monitoring surveys in Massachusetts and Cape Cod Bays. Undifferentiated GPS will be used routinely to achieve an accuracy better than 100 m. The latitude/longitude of the LORAN system will be automatically calibrated by the GPS position. Before each survey, the Northstar will be calibrated at Battelle at a known position having an accuracy of 5 m. The Northstar calibration will be checked daily at the dock before departure and after return to the dock.

11.1.2 Precision

LORAN time delays (TDs) and Northstar's calculated latitude/longitude positions will be recorded. The Northstar system automatically applies additional secondary-phase-factor (ASF) corrections to the LORAN data and the unit displays TDs in hundredths of microseconds and positions in 32 hundredths of a meter, providing accurate and repeatable positions.

11.1.3 Completeness

The Northstar will output navigation positions at an interval of 2 s. The BOSS software system will display all position fixes and save these fixes in an electronic file during all sampling operations (e.g., otter-trawl towing). The project's time interval requirement for obtaining positions during sampling is 1 min. Thus, even with a few bad data streams from the Northstar to the computer, the software will provide enough fixes within each 1-min period for 100% data collection. During transit between sampling sites, the BOSS software system will save vessel coordinates in an electronic file every 5 min.

11.1.4 Comparability

The Massachusetts Bay, Cape Cod Bay, and Boston Harbor charts will be displayed on the navigation CRT monitor. Corrected latitude/longitude positions will be recorded, and these positions will be comparable to those obtained by other researchers that have used or are using undifferentiated GPS or corrected LORAN.

11.1.5 Representativeness

The LORAN TDs and 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/longitude represent the actual vessel position.

11.2 Flounder Collection (Task 14)

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 10 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 10 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, including five males and five females, from each sampling site. Otter-trawl tows will be conducted until at least 50 specimens, including five males and five females, 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 Chief Scientist will determine the need for appropriate corrective action (e.g., resampling using a different otter trawl). The corrective action taken by the Chief Scientist will be recorded in the Survey Notebook.

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 15 min 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 15)

At each station, 10 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 Chatillon fish 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

The precision of the weights of the lobster will be enhanced by using a scale (Chatillon fish scale) with a maximum reading pointer (MRP) that retains the weight reading of the lobster until another lobster is put on the scale. 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, 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 in (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.

11.3.3 Completeness

The sampling objective is to obtain 10 commercially harvestable specimens representative of their location. Lobster pots will be set repeatedly at each sampling site until 10 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 10 lobsters are collected from each sampling site. In the event of sample loss or equipment malfunction, the Chief Scientist will determine the need for appropriate corrective action. The corrective action taken by the Chief Scientist will be recorded in the Survey Notebook.

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 Histological Data (Task 16)

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.

11.4.1 Accuracy

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

11.4.2 Precision

Not applicable

11.4.3 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 prevalences, lesion scores from six slides from each of 50 flounder livers from each site will be calculated. Three slides from each liver would be sufficient to provide the data for the statistical analyses, but there is no reason to believe that there will not be enough material to produce all six slides.

11.4.4 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 and otoliths 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. Michael Moore to assure that observations are comparable to those made during studies conducted previously.

11.4.5 Representativeness

Not applicable

11.5 Tissue Chemical Data (Task 17)

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, the recovery of 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. The QC samples will be analyzed as follows:

Organics:

- Every batch of samples will contain an SRM sample.
- 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 be noted in the laboratory records associated with analytical batches and in the project files. All QC data will be reported with the sample data. The goal for organics and metals QC analytical results (accuracy) is $\pm 30\%$ difference between the calculated

concentrations and the certified values (or known values, in the case of matrix spikes) for each individual analyte when concentrations are at least 10 times the analytical detection limits (in SRM samples only). 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%. 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 subtask leader to determine the necessity of reextraction or reanalysis. 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.

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 duplicate or MS/MSD samples is 30%. The RPD is calculated by

$$RPD = [\, 2\,(D_1 - D_2) \,/\, (D_1 + D_2)] \times 100$$

where D_1 = concentration of the first duplicate sample and
D_2 = concentration of the second duplicate sample.

Quality control criteria for accuracy and 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. All samples will be analyzed for the parameters listed in Table 5 and these analyses will be documented in the Chemistry Department project files.

Table 7. Polychlorinated Biphenyls/Pesticides Method Detection Limits

Analyte	*Tissue* (ng/g dry wt.)	*Analyte*	*Tissue* (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
$CL_3(28)$	0.81	$CL_6(153)$	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	$CL_7(170)$	0.79
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		

Table 8. Polynuclear Aromatic Hydrocarbons Method Detection Limits[a]

Analyte	*Tissue* (ng/g dry wt.)	*Analyte*	*Tissue* (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		

[a]Alkylated PAH will be assigned corresponding parent PAH Method Detection Limit.

Table 9. Metals Method Detection Limits (Processed Mass 0.5 g dry weight)

Tissue	*MDL* (µg/g dry wt.)	*Tissue*	*MDL* (µg/g dry wt.)
Al	0.5	Hg	0.025
Ag	0.025	Ni	0.5
As	0.05	Pb	0.1
Cd	0.025	Se	0.05
Cr	0.05	Sn	0.1
Cu	0.25	Zn	0.1
Fe	0.5		

Completeness of chemical analyses will depend directly upon the amount of sample available. A minimum of 5 g (wet weight) of tissue is necessary to perform all of the required analyses. 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 client 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

Standard reference materials (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. To accomplish this, Battelle participates in a series of interlaboratory calibration exercises for analysis of PAHs, PCBs, pesticides, and metals in sediments and tissues. Materials and instructions for organics analyses are provided by NIST. The National Research Council of Canada (NRCC) provides materials and instructions for metals intercomparison exercises.

Additionally, the methods used by earlier MWRA studies (1989-1992) 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***
Procedural Blank (Organics): 1/batch	< 5*MDL	Reextraction, reanalysis, and/or blank subtraction - determined by subtask leader; all corrective actions documented
Method Blank (Metals): 1/batch	< IDL	Blank subtraction determined by subtask leader
SRM Organics (PAH)*: 1/batch Metals: 1/batch	± 30% difference vs. certified values	Reextraction, reanalysis, and/or blank subtraction - determined by Task Leader; all corrective actions documented
MS/MSD Organics: 1 set per batch **Duplicates** Metals: 1 set per batch	50-150% recovery ≤ 30% RPD Average % CV: ± 35% individual analyte ± 30% average of all analytes	Document deviations
SIS (Organics): Every sample	50-150% recovery (one PAH SIS may exceed)	Results examined by project management or Project Area Leader. Corrective action (reextraction, reanalysis) or justification documented.
<u>Calibrations:</u> Initial	Organics: ± 25% RSD individual analyte ± 10% RSD average of all analytes Metals: ± 15% of true value .99 calibration correlation (r)	Reanalyze or document and justify
Check	Organics: ± 25% RSD individual analyte ± 10% RSD average of all analytes Metals: ± 15% of true value	Remedial maintenance, new initial calibration, reanalyze samples at discretion of analyst and task leader. Decision documented and/or justified.

* Certified values for NIST SRM 1974 are available only for PAH. Concensus values are available for PCB and pesticides.

11.5.5 Representativeness
Not applicable

12. 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 the BOSS 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. The GPS/LORAN system will automatically choose between GPS and LORAN, based on best accuracy.

The BOSS software acquires data from all onboard electronic sampling systems and navigation systems. The software queries each system four times per second. The software displays all of the information once per second on a color monitor. The navigation portion of the display will show the coastlines digitized from standard NOAA charts, navigation aids, sampling sites, and vessel track. Navigation data will be written to a navigation data file with event marker numbers added to document survey activities. A second monitor, if required, will be furnished to the helmsman as a steering display. During otter-trawl towing or lobster-pot deployment operations, position fixes will be electronically recorded at 2-s intervals. Hard-copy printouts of position fixes will be made during discrete sampling events such as triggering the start of the otter-trawl towing or deploying a lobster trap. During transit operations between stations, position fixes will be electronically recorded at 5-min intervals.

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 min 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 65 specimens have been collected at each sampling site. At the start and completion of each tow, the event will be electronically flagged in the BOSS data file using an unique event "marker_no" so that a precise vessel position and the concurrent parameters (e.g., time) can be linked to a particular flounder sample.

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 aftdeck 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 are not collected after four tows, 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. However, if any lobster specimens are collected in the otter trawl, they will be retained and processed according to steps described below.

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 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 weight and lengths as well as other information will be recorded on the flounder sample collection log (Figure 4). A bar-coded label will be attached to the log.

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Place label here

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)
									liver: chem__, hist__ scales__ tissue__
									liver: chem__, hist__ scales__ tissue__
									liver: chem__, hist__ scales__ tissue__
									liver: chem__, hist__ scales__ tissue__
									liver: chem__, hist__ scales__ tissue__
									liver: chem__, hist__ scales__ tissue__
									liver: chem__, hist__ scales__ tissue__
									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)

Figure 4. Winter Flounder Sample Collection Log.

12.2.2 Tissue Sample Processing

Processing will be conducted in the laboratory for the 10 fish for tissue chemistry analysis and on board the collection vessel for the 40 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, 10 fish of the proper size (age) and sex (5 males and 5 females) will be designated for tissue chemical analysis. The fish will be kept alive in separate containers, identified by site, until they are returned to the laboratory.

In the laboratory, 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 flounder tissues will be removed in the laboratory under contaminant-free conditions. Tissue processing will be conducted in a Class 100 clean room. Using a pre-cleaned (i.e., rinsed with 10% HCL, Milli-Q (18 megaohm) water, acetone, DCM, and hexane) titanium knife, the filets (muscle) will be removed from the flounder and the skin will be removed from the filet. Composites will be made using approximately equal masses of top and bottom tissue. The tissues will be placed in a pre-cleaned vessel (cleaned for organic and inorganic analyses) and homogenized by using a titanium tissuemiser; subsequently, the sample will be split for analyses. Each tissue sample will be placed in a sample container clearly identified with a bar-coded label containing the unique sample identifier (pathology laboratory accession number for the flounder liver) that will allow the sample to be traced from collection through analysis to reporting.

Chemical analysis of tissues will not be conducted until a histological analysis of the livers is first conducted. Based on the extent of disease observed in the livers, the histology subtask leader will assign the livers to three disease categories — severe, moderate, slight — thereby creating three pools of liver samples. If the histological analyses do not support such a categorization, the livers will be divided randomly into three equal (by weight) pools. The histology subtask leader will provide the chemistry subtask leader with the sample identifications of the livers for each pool.

Fish for Histology Analyses. The following steps refer to the 40 fish that will be processed on board the vessel.

1. The remaining 40 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 — 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. The heads of specimens >35 cm will be frozen and stored. The otoliths from selected specimens will be removed in the laboratory if NMFS notifies Battelle that the otoliths are needed to verify an age determined from the scales. Otoliths will be taken by slicing into the head of the flounder along an imaginary line extending from the end of the bony ridge between the eye and the edge of the gill cover until the bone is penetrated. The nose of the fish is bent down to open the head until one otolith is exposed. The other otolith is found directly beneath the first otolith. Otoliths will be removed from the fish with tweezers and will be placed between the liner in the age-sample envelopes containing a bar-coded sample label.

4. The livers will be removed and examined for visible gross abnormalities. They will be preserved in 10% neutral buffered formalin for histological analysis. Each liver sample 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 overnight 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 event will be electronically flagged in the BOSS data file using an unique "marker_no" so that a precise vessel position and the concurrent parameters (e.g., time) can be linked to a particular lobster sample.

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. Ten 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.

12.3.2 Laboratory Processing

Because it is unlikely that contaminant-free conditions will be found on board the vessel used for lobster collection, the lobsters will be returned to the laboratory for organ dissection. 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 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 5). A bar-coded label will be attached to the log. 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 label.

Data on external abnormalities will be used to separate the hepatopancreas and edible tissue into three pools each. These pools will be based on the severity of the external abnormalities — severe, moderate, slight. If the external examination does not support such categorization, the samples of hepatopancreas and edible tissue will be randomly divided into three equal (by weight) pools. The histology subtask leader will provide the chemistry subtask leader with the sample identifications of the various tissues for each pool.

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Place label here

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)
								liver___ tissue ___
								liver___ tissue ___
								liver___ tissue ___
								liver___ tissue ___
								liver___ tissue ___
								liver___ tissue ___
								liver___ tissue ___
								liver___ tissue ___
								liver___ tissue ___
								liver___ tissue ___

Entered by:_____ **Date:**_____

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

Figure 5. Lobster Sample Collection Log.

12.4 Histological Analysis (Task 16)

Livers of 10 flounder collected for chemical analysis from each of the five sites will be examined histologically before the remaining 40 flounder collected specifically 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 Task 14. If the scales taken from fish that are >35 cm do not provide a clear indication of age, a reading from the otoliths of that particular fish will be made.

Transverse sections of flounder livers fixed as part of Task 14 will be removed from the buffered formalin after at least 24 h, 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 two levels, with skip sections between, resulting in six slides per fish and a total of 1500 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 six slides.

12.5 Tissue Chemical Analyses (Task 17)

The chemical analytes of interest for Task 17 are listed in Table 5. Table 2 summarizes the analyses of tissue samples collected under Tasks 14 and 15. Three pools each of flounder samples (liver and edible tissue) and lobster samples (hepatopancreas and edible tissue) will be analyzed (see section 12.2 and 12.3 for details on pooling). Samples assigned to each specific pool will be homogenized together prior to conducting analyses.

12.5.1 Organic Analyses

Tissue samples will be serially extracted for PAH, chlorinated pesticides, and PCB following methods developed by Battelle in support of the NOAA Status & Trends Mussel Watch Project. 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 min 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 min, 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 or lobster hepatopancreas 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 or lobster 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). Pesticides and PCB congeners will be analyzed and quantified using gas chromatography/electron capture detection.

12.5.2 Metal Analyses

The tissue samples will be homogenized with an OMNI Tissue Homogenizer fitted with a titanium cutting probe. After homogenization, samples will be freeze-dried and digested with hot acids for dissolution and analysis of metals by atomic absorption spectrophotometry.

Sample processing details for inorganic analysis are described below. A 0.5-g subsample of the dry tissue is heated by microwave in a Teflon digestion vessel containing 6 mL HNO_3 and 0.5 mL $HClO_4$. The digestion solution is then diluted to $\approx$ 30 mL and transferred to pre-cleaned polyethylene storage containers for final analysis by atomic absorption spectrometry. Silver (Ag), cadmium (Cd), chromium (Cr), nickel (Ni), copper (Cu), lead (Pb), iron (Fe), aluminum (Al), and zinc (Zn) concentrations will be measured using graphite-furnace atomic absorption spectrophotometry (GFAAS). Mercury (Hg) will be measured in the digestion solution using cold-vapor atomic absorption spectrophotometry (CVAAS) using an LDC mercury monitor.

13. SAMPLE CUSTODY

13.1 Navigation Data

Field custody of electronic data will be the responsibility of the primary BOSS software operator for a specific survey. This person will be identified in each survey plan. The field custody of the electronic data consists of creating floppy-disk back-ups of all electronic data generated each day. Each floppy disk label will include a survey ID, date, name of person creating the backup files, and a disk number. When the equipment is returned to Battelle's laboratory, a second complete backup, labeled as "set 2," will be generated on floppy disks. "Set 2" will be in the custody of Mr. Carl Albro.

13.2 Flounder and Lobster Samples

Before the field surveys, a checklist of all samples to be collected in the field is 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 and lobster samples collected for Tasks 16 and 17.

Field samples are identified by a unique sample ID which is a concatenation of *event_id* (5-character ID unique to each survey) and *marker_no* (which is a non-repeating number for each survey generated by the BOSS software). The *event_id* that will be used for these surveys is F9301 (1993 survey) and F9401 (1994 survey). This *marker_no* will be created by BOSS when an otter-trawl tow is completed or a lobster-pot trawl is retrieved. This action will be electronically flagged in the BOSS data file using an unique *marker_no* so that the following pertinent information can be recorded:

Table 11. Sampling Protocols for Flounder/Lobster Surveys.

	Flounder			*Lobster*	
Station	***Liver***	***Edible Tissue***	***Scales/ Otoliths***	***Hepato-pancreas***	***Edible Tissue***
01	FLC, FLH	FTC	FSA	LHC	LTC
02	FLC, FLH	FTC	FSA	NA	NA
03	FLC, FLH	FTC	FSA	NA	NA
04	FLC, FLH	FTC	FSA	LHC	LTC
05	FLC, FLH	FTC	FSA	LHC	LTC

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

- Event_no
- Marker_no (tow)
- Date and time of event (starting)
- Otter trawl or lobster pot tow number
- Vessel coordinates at time of event (starting)
- Gear
- Sampling site
- Specimen number
- Protocol code
- Water depth

The software electronically saves this information in a log file. For each *marker_no* (i.e., otter-trawl tow or lobster-pot trawl), the BOSS program will generate several bar-coded labels that will be uniquely identified by a sample identification number that is comprised of the event number (survey) and number of the activity (e.g., otter trawl 1 would have a marker no = 001). Each specimen will be assigned a unique sample identification and specimen number. The bar-coded sample labels will be attached to sample containers, the chain-of-custody (COC) form, BOSS electronic data, and sample collection log. The ending coordinates (latitude and longitude), date, and time will be recorded electronically in the station log, but they will not appear on the bar-coded label.

Based on the types of samples that will be collected, COC forms will be generated for each sample type to track samples from collection to laboratory. Four COC forms will be generated for flounder samples; two COC forms will be generated for lobster samples. Figures 6a through 6f present examples of COC forms that will be used. Manual entries will be recorded in indelible ink in the data section of the COC only if the bar-coded label is destroyed or damaged.

CHAIN-OF-CUSTODY RECORD		
For: Flounder Scales		
Project Name: Harbor and Outfall Monitoring — MWRA Contract No. S138		
Sampling Site: Weather:		Date: Recorded by:
Date:		Tow:
Time:		Protocol:
Event ID:		Specimen No:
Marker No:		Comment:
Longitude:		
Latitude:		
Date:		Tow:
Time:		Protocol:
Event ID:		Specimen No.:
Marker No:		Comment:
Longitude:		
Latitude:		
Date:		Tow:
Time:		Protocol:
Event ID:		Specimen No.:
Marker No:		Comment:
Longitude:		
Latitude:		
Date:		Tow:
Time:		Protocol:
Event ID:		Specimen No.:
Marker No:		Comment:
Longitude:		
Latitude:		
Released By/Date/Time/Company	Transporter/Airbill #	Received By/ Date/Time/Company
PLEASE RETURN THE ORIGINAL OF THIS COMPLETED CHAIN-OF-CUSTODY FORM TO ROBERT HILLMAN—BATTELLE		

Figure 6a. Chain-of-Custody Form for Flounder Scales

CHAIN-OF-CUSTODY RECORD		
For: Flounder Histology Samples		
Project Name: Harbor and Outfall Monitoring – MWRA Contract No. S138		
Sampling Site: Weather:	Date: Recorded by:	
Date:	Tow:	
Time:	Protocol:	
Event ID:	Specimen No:	
Marker No:	Comment:	
Longitude:		
Latitude:		
Date:	Tow:	
Time:	Protocol:	
Event ID:	Specimen No.:	
Marker No:	Comment:	
Longitude:		
Latitude:		
Date:	Tow:	
Time:	Protocol:	
Event ID:	Specimen No.:	
Marker No:	Comment:	
Longitude:		
Latitude:		
Date:	Tow:	
Time:	Protocol:	
Event ID:	Specimen No.:	
Marker No:	Comment:	
Longitude:		
Latitude:		
Released By/Date/Time/Company	Transporter/Airbill #	Received By/ Date/Time/Company
PLEASE RETURN THE ORIGINAL OF THIS COMPLETED CHAIN-OF-CUSTODY FORM TO ROBERT HILLMAN–BATTELLE		

Figure 6b. Chain-of Custody Form for Flounder Scales.

CHAIN OF CUSTODY RECORD - INORGANIC CHEMISTRY ANALYSES

PROJECT NAME: MWRA Fish and Shellfish Task
PROJECT NUMBER:
BATCH NUMBER:
MATRIX:

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

Figure 6c. Chain-of Custody Form for Flounder and Lobster Inorganic Chemistry Analyses.

CHAIN OF CUSTODY RECORD - ORGANIC CHEMISTRY ANALYSES

PROJECT NAME: MWRA Fish and Shellfish Task
PROJECT NUMBER:
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 6d. Chain-of Custody Form for Flounder and Lobster Organic Chemistry Analyses.

CHAIN-OF-CUSTODY RECORD	
For: Lobster Chemistry Samples	
Project Name: Harbor and Outfall Monitoring – MWRA Contract No. S138	
Sampling Site: Weather:	Date: Recorded by:
Date:	Tow:
Time:	Protocol:
Event_ID:	Specimen No:
Marker_No:	Comment:
Longitude:	
Latitude:	
Date:	Tow:
Time:	Protocol:
Event_ID:	Specimen No.:
Marker_No:	Comment:
Longitude:	
Latitude:	
Date:	Tow:
Time:	Protocol:
Event_ID:	Specimen No.:
Marker_No:	Comment:
Longitude:	
Latitude:	
Date:	Tow:
Time:	Protocol:
Event_ID:	Specimen No.:
Marker_No:	Comment:
Longitude:	
Latitude:	

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

PLEASE RETURN THE ORIGINAL OF THIS COMPLETED CHAIN-OF-CUSTODY FORM TO ROBERT HILLMAN–BATTELLE

Figure 6e. Chain-of Custody Form for Lobster Chemistry Samples.

CHAIN-OF-CUSTODY RECORD		
For: Flounder Stomach Samples		
Project Name: Harbor and Outfall Monitoring – MWRA Contract No. S138		
Sampling Site: Weather:	Date: Recorded by:	
Date: Time: Event_ID: Marker_No: Longitude: Latitude:	Tow: Protocol: Specimen No: Comment:	
Date: Time: Event_ID: Marker_No: Longitude: Latitude:	Tow: Protocol: Specimen No.: Comment:	
Date: Time: Event_ID: Marker_No: Longitude: Latitude:	Tow: Protocol: Specimen No.: Comment:	
Date: Time: Event_ID: Marker_No: Longitude: Latitude:	Tow: Protocol: Specimen No.: 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 ROBERT HILLMAN–BATTELLE		

Figure 6f. Chain-of Custody Form for Flounder Stomach Samples.

Each completed COC will be signed and dated by the staff member entering the information.

In addition, a station log will be maintained by the Chief Scientist in a project-specific Laboratory Record Book (LRB). Data that are collected for each station will be stored in electronic format. The station log includes pertinent information about each station, such as time on station, weather observations, marker_no data, and general comments by the Chief Scientist.

The COC forms will accompany samples to their final destination. If the custody of samples is transferred, the staff member relinquishing custody of the samples will enter the shipment method, preservative, date and time, and sign the form in the *Released by* or *Relinquished by* area. The staff member assuming custody of the samples will verify that all samples listed on the COC are present, and he will enter the date and time of receipt of the samples and sign the form in the *Received by* area. 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. Copies of all COC forms will be returned to Battelle's fish and shellfish monitoring Project Area Leader.

Field custody of samples will be the responsibility of the Chief Scientist, Ms. Deborah West. She will ensure that bar-coded sample labels are attached to samples and to the COC forms. In addition, the field custodian ensures that samples are stored properly and safely until custody is relinquished. Ms. West will also be the custodian of the navigation data.

13.3 Histology Samples

The laboratory custodian of samples for histological analyses will be Ms. Joanne Lahey. The laboratory custodian of age samples will be Ms. Karen Foster. Age samples will be tracked at the NMFS laboratory by the bar-coded labels on the envelope in which the age samples are stored. This envelope will also contain the accession number (identification number) of the specimen. Ages of fish will be written on the envelope to eliminate transcription errors.

When the livers are removed and placed in fixative, each liver will receive a Battelle Pathology Laboratory accession number which identifies the fish through final slide preparation. Accession numbers are assigned chronologically by year (e.g., 93-XXXX). The first of the transverse sections from each liver will be identified by the letter *A*, the second by the letter *B*, and the third by the letter *C*. The first slide cut from section A will be numbered *1* and the second slide will

be numbered *2*. Thus, a slide from the first transverse section could have a number 93-1234A1. This number, which is also on a slide label, is entered into the laboratory slide log book with the site data. The log book is maintained in the pathology laboratory. Slides are archived in a slide file not within the confines of the laboratory.

13.4 Samples for Tissue Chemistry

The laboratory custodian of samples for organic analyses will be Mr. Daniel Bardon. The laboratory custodian of samples for inorganic (metal) analyses will be Ms. Roxanne Brackett. They will be responsible for receiving samples (by signing the COC) 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. CALIBRATION PROCEDURES AND PREVENTIVE MAINTENANCE

Logs of maintenance, calibrations, and any repairs made to instruments will be stored in the instrument files kept by Battelle. Maintenance of and repairs to instruments will be in accordance with manufacturers' manuals.

14.1 Navigation Equipment

The GPS receiver (integrated in the Northstar GPS/LORAN-C system) will provide a corrected latitude/longitude position that will be used to automatically calibrate the LORAN latitude/longitude when GPS data are not being received. Positions will also be checked at certain fixed calibration points; the absolute positions of these calibration points will be obtained from published charts and light lists. The time and position of the calibration sites will be printed out by the BOSS software and entered in the BOSS Survey Notebook. Following the survey, all navigation calibration information will be assessed to determine whether it is necessary to apply a calibration adjustment to the LORAN positions. This will be documented in the Chief Scientist's notebook.

The Northstar GPS/LORAN-C system automatically undergoes a thorough self-test when turned on. Cable connections and antenna mounts and brackets will be

inspected and secured at regular intervals. The system will be maintained according to manufacturer's instructions.

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. IN-4, 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 instrumentation will be properly calibrated and maintained in accordance with SOPs or manufacturer instructions as specified in operations manuals. Procedures for calibration and maintenance of the more complex analytical equipment are described below.

Analytical instruments will be calibrated before sample analysis. Response factors (RFs) 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

Three 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) of the RFs are within 25% of the mean for each individual analyte, and the mean of all analyte RSDs is 10%. Any exceptions will be documented and justified by the subtask leader.

The system calibration will be verified a minimum of once every 24 h by using a mid-range calibration check. Using the mean RF of each analyte from the initial calibration, the percent difference between the mean values and the RFs from the mid-range 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
RF_r = response factor from the mid-range calibration check.

The calibration checks will be acceptable under the same criteria as the initial calibration (i.e., 25% for individual analytes, 10% 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 will be reanalyzed, at the discretion of the analyst and project management. Because gas chromatography with electron-capture detection (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. Reanalyses will be performed at the discretion of the analyst and subtask leader. This decision will be documented in the project files.

Samples analyzed by GC/ECD, GC/FID, 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.

Detector response (electron-capture detectors, flame ionization 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, coprostanol calibrations). Both the responses per unit amount and the resolution of specific components will be monitored. If any evidence of deterioration in the performance of the chromatographic column is observed, the column will be replaced. In addition, a log containing a detailed record of all maintenance performed will be maintained for each instrument.

14.3.2 Instrumentation for Metals Analysis

The atomic absorption instrumentation will be calibrated daily before samples are analyzed. Calibration standards will be prepared each day and tissue sample digestion solutions will be quantified by GFAAS using the method of additions calibration to avoid inaccuracies resulting from chemical interferences. Calibration standard check samples (as NIST certified aqueous sample 1643c or EPA performance evaluation samples) will be analyzed every 10 samples to ensure continued accuracy. Measurements that are not bracketed by an accuracy check standard within 15% of its true value will be rejected and reanalyzed after corrective action is taken (as needed). GFAAS measurements will be made in duplicate for each sample; if the relative percent difference (RPD) between duplicate injections is greater than 10%, then the sample measurement will be rejected unless the absorbance values are very low and small differences (<0.004 abs units) result in high RPD values. Sample quantitations will only be accepted if the standard additions quantitation curve has a correlation coefficient of 0.99 or better.

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 15% acceptance criteria), prepared by an analyst other than the analyst that prepares the calibration standards (aqueous mercury SRMs are not currently available for mercury). 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 for the calibration curve is not obtained for the atomic absorption instrumentation, then the instrument operation and instrument integrity will be assessed and analytical standards evaluated. 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 atomic absorption standard by more than 10% will be reanalyzed (after appropriate dilution if necessary). All instrumental maintenance will be documented in instrument log books.

Maintenance of the GFAAS instrumentation includes daily cleaning of the furnace contacts and windows, and daily inspection of graphite tube and stabilized temperature platform. Use/maintenance log forms are filled out daily when in use for each metal so that cleaning activities and lamp performance are documented. Battelle maintains manufacturer service contracts for GFAAS instruments and instrument hardware failures are attended by Perkin-Elmer Corporation service personnel as needed.

The mercury instrumentation maintenance may include changing the mercury lamp, cleaning the absorption cell, or cleaning the instrument electrical contacts. Instrument use and maintenance activities are recorded daily when in use on use/maintenance log forms located adjacent to the mercury instruments.

15. DOCUMENTATION, DATA REDUCTION, AND REPORTING

15.1 Data Recording

All data will be initially recorded either (1) electronically onto computer storage media from BOSS or other laboratory system or (2) manually into bound laboratory notebooks or (3) manually onto 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. Changes will be made by drawing a single line through the original entry, initialing, dating, and explaining the change. It will be the responsibility of the laboratory managers to ensure that all data entries and hand calculations are verified in accordance with procedures described in Section 16. In addition to these documentation procedures, sample logs associated with field and laboratory custody and tracking will be maintained by Dr. Hillman in project files located in his office.

The BOSS data-acquisition software assigns a unique data filename to each otter-trawl tow made or lobster-pot trawl deployed during the survey. The time, date,

and position of the start and end of each data file are automatically entered into the electronic log book that is stored on a computer hard disk. A hardcopy printout is simultaneously created. The printouts will be punched and inserted into the BOSS survey notebook. At the end of each tow or when a lobster trawl is retrieved, the graphic screen of navigation data will be saved to a file on the computer hard disk. On each survey, the screen dumps will be printed in color on the HP PaintJet and stored in the BOSS survey notebook which will be in the custody of Ms. West.

15.2 Data Reduction

15.2.1 Navigation Data

The vessel otter-trawl tracks and lobster-pot deployment positions 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 six 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 1993 to 1994.

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 by the Hewlett Packard 3350A Laboratory Automation System. All organics analytical data will be transferred into Oracle from which the final report tables will be generated. 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:

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

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, and between certified SRM values and calculated SRM values resulting from sample analysis will also 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 (or certified SRM value)
C_2 = concentration (ng/g) of analyte detected in sample 2 (or detected in SRM).

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

15.3 Reporting Data to be Loaded into the Database

Only data that have been designated by the Project Area Leader as final will be loaded into Battelle's copy of the Harbor Studies Database. All data submitted for inclusion in the Harbor Studies Database will adhere to the formats described below.

15.3.1 Navigation and Sample Collection Data

Navigation and sample collection data contained in the BOSS electronic log file will be provided as Lotus spreadsheet files after completion of the survey report. The columns will include sample_id, site_id, water_depth, date, time, latitude, longitude, and protocol code.

15.3.2 Analytical and Experimental Data

Data from chemical (organic and metal) analysis and histological analysis of flounder and lobster samples will be submitted in Lotus spreadsheets. The format for these data is presented in Figure 7 and 8 (Task 16) and Figure 9 (Task 17).

15.4 Loading Data into the Harbor Studies Database

Data provide by Battelle will be loaded into the Harbor Studies Database by Battelle data management staff. Upon receipt of data from subtask leaders, each diskette will be logged in and assigned a unique login identifier. Any changes or additions to data, necessary for loading into the Harbor Studies Database, will be made using SQL scripts. The original diskette, SQL scripts, and data-loading documentation will be filed according to login identifier at Battelle. The data sources notebook will contain copies of the COC forms, the MWRA data documentation form, and data entry information.

16. 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. Battelle Project Area Leaders will be responsible for validation of all data generated. Validation procedures for data generated by Battelle will include the following:

- Data that are hand-entered into a database or spreadsheet will be either verified 100% for accuracy or will be entered in duplicate and compared electronically to identify differences.

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

- Calculations performed by software will be checked by the technical staff member at a frequency sufficient to ensure the accuracy of the calculations.

- Electronically generated data will be reviewed in graphical form by the technical supervisor to ensure that the data are complete, accurate, and technically reasonable. The removal of all outliers, either manually or by computer algorithm, will be reviewed in graphical form by the technical supervisor.

MWRA HARBOR STUDIES DATABASE

Data Reporting Format: Histopathology
Date: February 26, 1993
Contact: John Hennessy
Battelle Ocean Sciences (617) 934-0571

Column	Type	Size	Must Report	Description
Sample_ID	Char	10	Y	Unique sample ID assigned during field collection (i.e., number on bar-coded label).
Spec_code	Char	12	Y	NODC code for organism
Organ	Char	10	Y	Organ being studied
Histo_var	Char	8	Y	Histopathological variable of interest
Hist_val	Char	10	Y	Value for histopathological variable
Lab_sample_id	Char	35	Y	Slide number
Comments	Char	150	Y	Comments

Notes:
1) A column to report value qualifier (val_qual) will be added if appropriate.
2) Hist_val is a character variable because of the flexibility of being able to put a numeric in the character field, whereas a character cannot be put in a numeric field.

Figure 7. Spreadsheet Format for Reporting Histological Analysis Results.

Histo_var Codes	Description	Hist_val Codes	Hist-val Code Description
YR	Age of fish in years		
SEX	Gender	F M	Female Male
TL	Total length (mm)		
FIN	Fin-rot score	0 1 2 3 4	Absent Minor Moderate Severe Extreme
GR	Gross lesions	0 1 2 3 4	Absent Minor Moderate Severe Extreme
GS	Gross score	0 1 2 3 4	Absent Minor Moderate Severe Extreme
LC	Liver color	Y YB B DB G	Yellow Yellow brown Brown Dark brown Green
MN	Mean histological index for that lesion type	0 1 2 3 4	Absent Minor Moderate Severe Extreme
P1	Prevalence score (from 1 tissue piece)	0 1	Absent Present
P3	Prevalence Score (from 3 tissue piece)	0 1	Absent Present
CHV	Centrotubular hydropic vacuolation	0 1 2 3 4	Absent Minor Moderate Severe Extreme
THV	Tubular hydropic vacuolation	0 1 2 3 4	Absent Minor Moderate Severe Extreme
FHV	Focal hydropic vacuolation	0 1 2 3 4	Absent Minor Moderate Severe Extreme
MA	Macrophage aggregation	0 1 2 3 4	Absent Minor Moderate Severe Extreme
BDP	Biliary duct proliferation	0 1 2 3 4	Absent Minor Moderate Severe Extreme
NEO	Neoplasia	0 1 2 3 4	Absent Minor Moderate Severe Extreme

Figure 8. Codes for Histopathological Variables.

MWRA HARBOR STUDIES DATABASE

Data Reporting Format: Analytical Results (Battelle Chemistry)
Date: January 29, 1993
Contact: John Hennessy and Lynn Lariviere
Battelle Ocean Sciences (617) 934 - 0571

Column	Type	Size or Precision	Scale	Must Report	Description
Sample_ID	Char	10		Y	Unique sample ID assigned during field collection (i.e., number on bar-coded label).
Lab_sample_ID	Char	8		Y	Identifier assigned to sample by analytical laboratory
Param_code	Char	20		Y	CAS code for analyte
Anal_date	Date			Y	Date of sample extraction
Value	Number	12	5	Y	Resultant data value determined by analytical procedure
Unit_code	Char	10		Y	Code for value units
Inst_code	Char	5			Code for analytical instrument used to analyze sample
Val_qual	Char	3			Code that qualifies data value
Meth_code	Char	8		Y	Code for method used to analyze sample
QC_code	Char	4		Y	Code indicating type of sample (QC = QC sample, SAMP = Normal Sample)
Anal_rep	Number	3	0		Sequential number, beginning with 1, assigned to replicate analyses of a single sample
Batch_no	Char	10			Identifier assigned to batch of samples by the analytical laboratory (no spaces or punctuation marks)
Detect_limit	Number	9	5	Y	Detection limit
Comments	Char	30			Comments

Notes:

1. Data resulting from laboratory QC samples should be submitted along with your data package. For laboratory QC samples, no entry should be reported for Sample_ID but a Lab_sample_ID must be reported.
2. Codes are provided in the code list for the following fields: meth_code, inst_code, unit_code, val_qual, and param_code. If you require a code that is not in the code list, please contact us.
3. Precision is the maximum size of a number excluding the decimal point. Scale is the number of places after the decimal place. For example, 10.234 is stored with precision = 5 and scale = 3.

Figure 9. Spreadsheet for Reporting Tissue Chemistry Analyses Results.

- Analytical results and supporting data will be reviewed by the analytical supervisor to ensure that the data are complete, accurate, and technically correct prior to submission to the database.

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

- Subtask leaders will make professional judgements about the quality of suspicious individual data. Highly suspect data will be flagged as appropriate, and not included in any data analyses. However, filling data gaps will not be included as part of this project.

A primary component of data validation is verification that the documented procedures are in control. This review will be conducted at a frequency sufficient to implement corrective action (Section 18). QC samples that do not meet the data quality requirements will be documented as deviations in the corrective action log (see Section 18). The Project Area Leader will determine whether out-of-control QC results will invalidate or qualify data reported for field samples.

17. PERFORMANCE AND SYSTEMS AUDITS

This project will be monitored by the Task QA Officer under the direction of the Project QA Officer. The Task QA Officer will oversee the quality of the data generated during the project. All tabular and graphic data reported in deliverables and associated raw data generated by Battelle will be audited by the Task QA officer. Raw data will be reviewed for completeness and proper documentation. Statistical random audits of reported laboratory data will be conducted to ensure that the data are accurate, traceable, and comply with guidelines of the Harbor and Outfall Monitoring Project QAPP and with the QA specifications in this CW/QAPP. For electronically acquired data (e.g., BOSS data files), the Task QA Officer will verify that computer software used to process the data have been validated.

All deliverables generated during the course of this project will be submitted for an internal review prior to delivery of drafts to MWRA. This three-part process consists of technical, editorial, and QA reviews.

Laboratory inspections are performed by Battelle's Task QA Officer as part of the Harbor and Outfall Monitoring Project QAPP.

18. CORRECTIVE ACTION

Identification of problems related to technical performance is the responsibility of all staff members working on this project. Responsibility for identifying deviations from the schedule lies with the Battelle Project Manager. Technical problems relating to sample collection in the field (e.g., schedule, modifications to the sampling plan, etc.) will be resolved through discussion with the Battelle Chief Scientist, the Fish and Shellfish Project Area Leader, and the MWRA Project Area Manager. Problems relating to the overall successful completion of the project will be reported to the MWRA Project Area Manager in a timely manner for discussion and resolution between the Battelle and MWRA managers. Deviations from the survey and analytical program described in this CW/QAPP, or from the survey plans will be documented in the survey report.

Corrective action at the laboratory level will be performed by the laboratory staff. Any issues that affect the schedule or performance of the task will be reported to the Battelle Fish and Shellfish Project Area Leader or to the Battelle Project Manager. They will be responsible for evaluating the overall impact on the project and for discussing corrective actions with the MWRA Project Area Manager.

19. REPORTS

Reports that will be generated under Tasks 14, 15, 16, and 17 include survey plans and survey reports for each of the two surveys 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 one week 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 Histology Data Reports (Tasks 16 and 25)

Histological data reports (Task 16) 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 report (Task 25), and will include the following:

SUMMARY
CONCLUSIONS
RECOMMENDATIONS

INTRODUCTION
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

Information gathered by Woods Hole Oceanographic Institute under Task 3 will also be incorporated. With tables, the histopathology reports will be approximately 50 pages in length.

19.2 Tissue Chemistry Data Reports (Task 17)

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 25, specifically the fish and shellfish monitoring annual synthesis report (Task 25.2.3) and the annual toxics review (Task 25.5).

20. REFERENCES

Flescher, D.D. 1980. Guide to some trawl-caught marine fishes from Maine to Cape Hatteras, North Carolina. NOAA Technical Report NMFS Circular 431. U.S. Dept. of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service. 35 pp.

MWRA. 1991. Massachusetts Water Resources Authority effluent outfall monitoring plan. Phase I: baseline studies. MWRA Environmental Quality Department, November 1991. Massachusetts Water Resources Authority, Boston, MA. 95 pp.

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