Combined work/quality assurance project plan (CW/QAPP)

for

Fish and Shellfish Monitoring: 2004-2005

Massachusetts Water Resources Authority

Environmental Quality Department
Report ENQUAD ms-096
Version 1

Citation: Lefkovitz, L, SL Pala, and MJ Moore. 2004. **Combined Work/Quality Assurance Project Plan (CW/QAPP) for Fish and Shellfish Monitoring: 2004-2005**. Boston: Massachusetts Water Resources Authority. Report ENQUAD ms-096 Version 1. 34 p.

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

for

Fish and Shellfish Monitoring: 2004-2005

Tasks 21, 22, 23, 24 and 25

MWRA Harbor and Outfall Monitoring Project
Contract No. S366

Submitted to

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August 2004
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COMBINED WORK/QUALITY ASSURANCE PROJECT PLAN (CW/QAPP)

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FISH AND SHELLFISH MONITORING: 2004-2005

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Contract No. S366

Concurrences and Approvals

_____	_____
Ms. Ellen Baptiste-Carpenter Battelle Project Manager	Date
_____	_____
Carlton Hunt, Ph.D. Battelle Technical Director	Date
_____	_____
Ms. Rosanna Buhl Battelle Project QA Officer	Date
_____	_____
Dr. Michael Mickelson MWRA Project Manager	Date
_____	_____
Mr. Maury Hall MWRA Project Area Manager	Date
_____	_____
Ms. Wendy Leo MWRA EM & MS Manager	Date

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1.0 PROJECT NAME

Fish and Shellfish Monitoring (2004-2005)
Tasks 21, 22, 23, 24, and 25
MWRA Harbor and Outfall Monitoring Project

2.0 PROJECT REQUESTED BY

Massachusetts Water Resources Authority

3.0 DATE OF REQUEST

November 7, 2001

4.0 DATE OF PROJECT INITIATION

November 7, 2001

5.0 PROJECT MANAGEMENT

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Dr. Michael Mickelson, MWRA Harbor and Outfall Monitoring Project Manager
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Ms. Ellen Baptiste-Carpenter, Battelle Harbor and Outfall Monitoring Project Manager
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Mr. Chris Gagnon, Battelle Harbor and Outfall Monitoring Field Manager
Ms. Deirdre Dahlen, Battelle Harbor and Outfall Monitoring Laboratory Manager
Ms. Lisa Lefkovitz, Battelle Fish and Shellfish Monitoring Senior Scientist
Dr. Michael Moore, Woods Hole Oceanographic Institution, Senior Scientist

6.0 QUALITY ASSURANCE MANAGEMENT

Ms. Wendy Leo, MWRA EM & MS Manager
Ms. Rosanna Buhl, Battelle Quality Assurance Officer

7.0 PROJECT DESCRIPTION

7.1 Objectives and Scope

The Massachusetts Water Resources Authority (MWRA) is continuing a long-term biomonitoring program for fish and shellfish (MWRA 1991) for the MWRA effluent outfall that is located in Massachusetts Bay (see Figure 1). The goal of the biomonitoring is to provide data that may be used to assess potential environmental impact of effluent discharge into Massachusetts Bay. These data will be used to ensure that discharge from the new outfall does not result in adverse impacts by comparing values with established thresholds (MWRA, 2001).

The overall objective of the fish and shellfish monitoring is to define the condition of fish and shellfish health in terms of the presence of disease (external and internal), and organic and inorganic (metal) contaminant concentrations in the liver (winter flounder), hepatopancreas (lobster), and edible tissue (winter flounder, lobster and mussel) of these selected organisms. To help determine the body burden of toxic substances and to assess the physiological status of winter flounder (*Pseudopleuronectes americanus*), one survey will be conducted in Boston Harbor, Massachusetts Bay, and Cape Cod Bay (hereafter: Boston Harbor and Offshore) during 2004 and 2005 to collect specimens for analysis. Only morphological and pathological analyses (Tasks 21 and 25) are scheduled for 2004 and 2005; chemical analyses (Task 24) of the collected flounder tissue may be conducted at the discretion of the MWRA, as described in Version 0 of the Combined Work/Quality Assurance Project Plan (CW/QAPP) for Fish and Shellfish Monitoring: 2002 – 2005 (Lefkovitz *et al*., 2002). To determine body burden and physiological status of lobster (*Homarus americanus*) and blue mussel (*Mytilus edulis*), one survey per species will be conducted in Boston Harbor and Offshore every 3 years, with the next collection scheduled to occur in 2006. These surveys (Tasks 22 and 23) may be conducted during 2004 and 2005 at the discretion of the MWRA, as described in Version 0 of the CW/QAPP for Fish and Shellfish Monitoring: 2002 – 2005 (Lefkovitz *et al*., 2002).

With effluent discharge beginning in September 2000, results from these monitoring activities occurring post-2000 should alert MWRA to potential changes resulting from the relocation of the outfall discharge. The MWRA Contingency Plan (MWRA, 2001) specifies numerical or qualitative thresholds that may suggest that environmental conditions offshore may be changing or might be likely to change. The Plan provides a mechanism to confirm that a threshold has been exceeded, to determine the causes and significance of the event, and to identify the action necessary to return the trigger parameter to a level below the threshold (if the change resulted from effluent discharge). Fish and shellfish thresholds have been established for tissue contaminant concentrations (organic and inorganic) and liver disease incidence (MWRA 2001, Appendix A). Specific objectives for each of the tasks to be conducted in 2004 and 2005 are described in Sections 7.1.1 through 7.1.5.

This CW/QAPP presents the organization, objectives, functional activities, and specific quality assurance (QA) and quality control (QC) activities associated with the Fish and Shellfish Monitoring that will be conducted under Tasks 21 through 25 of the MWRA Harbor and Outfall Monitoring Program (Contract S366) in 2004 and 2005. This document also describes the specific protocols that will be followed for sampling, sample handling and storage, chain of custody, and laboratory and field analyses. The CW/QAPP was prepared in accordance with EPA guidance documents on CW/QAPP-preparation (EPA 1984, 1988) and is based on the CW/QAPP that guided previous Harbor and Outfall (HOM) fish and shellfish monitoring (Lefkovitz *et al*, 1998; Lefkovitz *et al*, 2001; Lefkovitz *et al*, 2002). Separate survey plans developed for each survey will supplement the CW/QAPP. The survey plans will provide the operational details required to conduct each survey, and will describe participating staff, schedule details, and specific equipment.

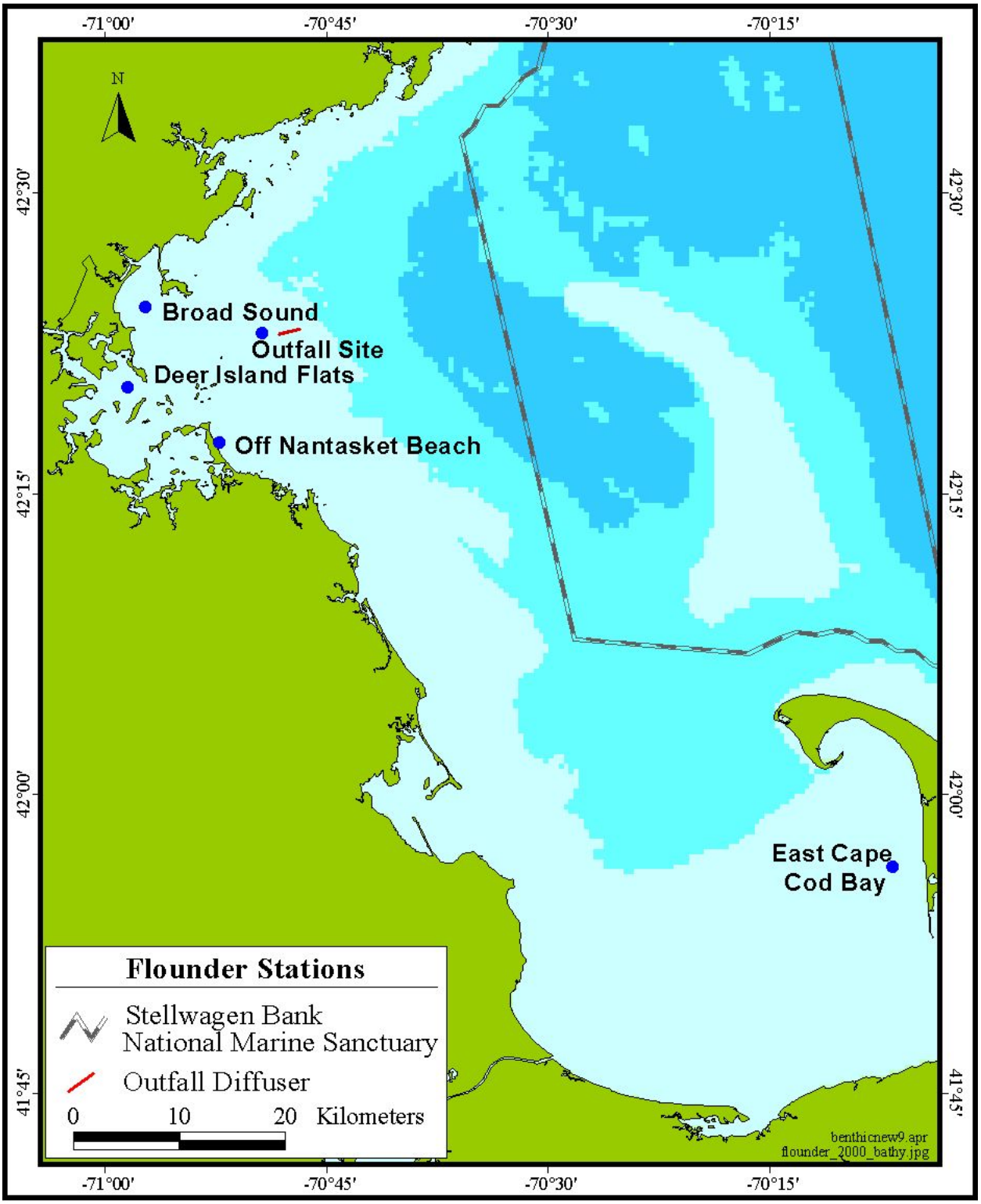

Figure 1. Flounder Monitoring Locations.

7.1.1 Flounder Survey (Task 21)

The objective of the survey is to obtain specimens of winter flounder *(Pseudopleuronectes americanus)* from five sampling sites in Boston Harbor and Offshore for gross examination, histology, aging, and chemical analyses of tissue to determine sublethal effects of contaminant exposure and tissue burden. Specimens will be collected during surveys conducted in April 2004 and 2005 for gross examination, histology, and aging; fillet and liver samples will be composited and archived for potential chemical analyses to be performed by Battelle. Chemical analyses (Task 24) are not scheduled until 2006 but may be performed in 2004 and 2005 at the discretion of the MWRA, based on the flounder histological results from Task 25. Any chemical analyses performed will follow the procedures described in Version 0 of the CW/QAPP for Fish and Shellfish Monitoring: 2002 – 2005 (Lefkovitz *et al*., 2002). Data generated will be evaluated against established thresholds.

7.1.2 Lobster Survey (Task 22)

The objective of the survey is to obtain specimens of lobster *(Homarus americanus)* from three sampling sites in Boston Harbor and Offshore for gross examination and chemical analyses of tissues to determine health and tissue burden of contaminants. The next lobster survey is scheduled for July 2006 and is not described in this CW/QAPP.

7.1.3 Mussel Bioaccumulation Survey (Task 23)

The objectives of the survey are to obtain, deploy, and recover blue mussels *(Mytilus edulis)* for determination of short-term accumulation of anthropogenic contaminants in mussel tissue. The next mussel bioaccumulation survey is scheduled for June-August 2006 and is not described in this CW/QAPP.

7.1.4 Tissue Chemical Analyses (Task 24)

The objective of tissue chemical analyses is to determine the body burdens of toxic substances and potential elevations of these body burdens caused by relocation of the outfall. Tissue chemical analyses are scheduled to be performed in 2006 but may be performed on the flounder tissue collected in 2004 and 2005 under Task 21 at the discretion of the MWRA. Any chemical analyses performed will follow procedures described in Version 0 of the CW/QAPP for Fish and Shellfish Monitoring: 2002 – 2005 (Lefkovitz *et al*., 2002).

7.1.5 Flounder Histological Analysis (Task 25)

The histological analysis is designed to assess the health of the flounder populations in Boston Harbor and Offshore by performing microscopic examinations of tissue sections of the flounder livers collected under Task 21. The bioeffects of contaminant exposure on 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 potential chemical analysis).

7.2 Data Usage

Histological data will be used to assess the sublethal effects of contaminant exposure and tissue burden of the flounder populations in the Boston Harbor and Bay areas sampled. Age data will be used to determine the age of the winter flounder examined for histology. Post-outfall relocation data will be compared with baseline data as part of the data evaluation.

Histological results collected after outfall relocation will be compared to baseline measurements and to threshold values (MWRA 2001, Table 1) to determine if the outfall relocation has had a measurable effect on the health of these organisms.

Table 1. Summary of Threshold Values for Flounder Histological Thresholds.

Organism	Threshold ID	Parameter	Unit of Measure	Threshold Value		Baseline years
				Caution	Warning	
Flounder	FFLIVDIS	liver disease incidence	%	44.94	-	1991-2000

7.3 Technical Approach

7.3.1 Flounder Surveys (Task 21)

A flounder survey will be conducted annually during April 2004 and 2005. A Survey Plan will be prepared for each of the annual surveys. The Survey Plan will detail sampling dates, locations, vessel, personnel, any deviations from the CW/QAPP, and general survey operations. These plans will be delivered to MWRA no later than two weeks before the start of each survey (i.e. March).

Five sites will be sampled during each annual survey to collect winter flounder for histological and potential chemical analyses (Figure 1):

- Deer Island Flats (Boston Harbor),
- Off Nantasket Beach (archived for chemistry in 2004 only),
- Broad Sound (archived for chemistry in 2004 only),
- Outfall Site (offshore effluent outfall),
- East Cape Cod Bay.

Table 2 provides the sampling sites and locations, although there may be differences of 1 km or more between collection sites and the indicated position due to the trawling operations. Adjustments in location will be made in the field to ensure that flounder are captured.

Table 2. Sampling and Locations for Flounder Surveys.

Station #	Sampling Site	Location	
		Latitude	Longitude
DIF	Deer Island Flats (Boston Harbor)	42°20.4'	70°58.4'
NB	Off Nantasket Beach	42°17.6'	70°52.2'
BS	Broad Sound	42°24.4'	70°57.2'
OS	Outfall Site	42°23.1'	70°49.3'
ECCB	East Cape Cod Bay	41°56.2'	70°06.6'

At each of the five designated sampling sites, otter-trawl tows will be conducted to collect 50 sexually mature, 30 – 50 cm (usually 4-5 year old) winter flounder (*Pseudopleuronectes americanus*). If unusually large (> 50 cm) flounder are obtained, up to 3 large individuals per site will be retained for processing. Each fish will be assigned a unique identification number to indicate the event, year, survey, and site of collection.

Fish destined for only histological analysis are killed at sea by cervical section and used for histological processing. These fish will be examined externally and their external condition will be noted prior to

histological processing. Guidance for recording external lesions in flounder is included in Appendix B. The gross external condition of the flounder ("External Lesions") and fin rot will be subjectively scored on a scale of 0 to 4. The gonads of each flounder will be examined to determine sexual maturity. All specimens will be weighed, and standard and total length (Figure 2) will be determined by measuring fish length according to Battelle Standard Operating Procedure (SOP) 5-175. Scales will be taken from each specimen for age determination. In addition, the liver will be aseptically removed, examined for grossly visible abnormalities, and preserved in 10% neutral buffered formalin. The presence of gross lesions on the liver will be subjectively scored on a scale of 0 to 4 and recorded as "Gross Liver Lesion".

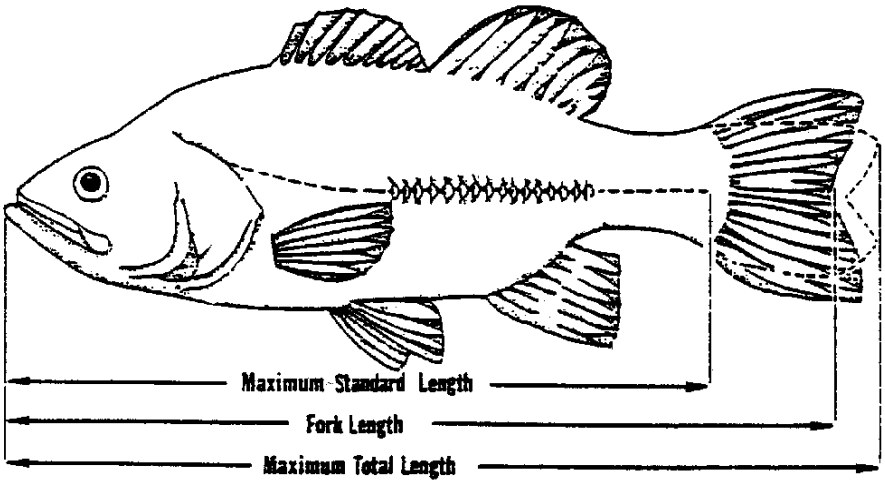

Figure 2. Length Measurements for Flounder.

For stations where tissue samples will be collected and archived for potential chemistry analyses, fifteen of the fifty fish will be randomly[1] selected, placed alive on ice, and transported to Battelle Duxbury Operations for on-shore processing for histological analysis and compositing for archival. Fifteen unique sample identification numbers will be assigned to these fish, however, actual assignment of IDs to individual fish will not occur until the fish are sacrificed at the laboratory. At this time, these fish will also be examined externally, their external condition will be noted (fin rot and external lesions), liver abnormalities noted, and scales removed for age analysis.

In the laboratory, fish will be killed by cervical section. Fillets (muscle) will be removed from the flounder, and the skin will be removed from the fillet. The livers will be removed and examined for visible gross abnormalities (gross liver lesion) as described above. A small section of the liver will be removed and preserved in 10% neutral buffered formalin for histological analysis. Fillet composites will be made from equal aliquots (±10% by weight) of the homogenate of 5 individual fish fillets using approximately equal masses of top and bottom tissue. The liver composite samples will contain approximately equal masses (5 grams) from each of the livers and will correspond to the composites made for the fillets[2]. The composites are then homogenized and stored frozen.

[1]"Random" in the context of this document means not consciously choosing or excluding specific animals. It is not meant to imply that animals were selected using a random numbers program.

[2] In cases where sample mass may be limited (i.e. flounder liver and lobster hepatopancreas), best professional judgment will be used when combining individual samples to form the composite sample so that enough composite sample is available to perform all of the required chemical analyses.

Within 2 and 30 days after each flounder survey, an e-mail Survey Summary and Report, respectively, will be prepared and submitted to the MWRA. The survey summary shall note completion of the survey and any noteworthy problems or events encountered. This summary will highlight any apparent triggering of monitoring thresholds, or conditions, which, if continued, might lead to such triggering. The report will include a summary of survey operations, number/species of specimens collected at each station for each discrete sampling event (e.g., each otter trawl), number of specimens dissected, observations made during sampling and dissection, and the disposition of the tissue samples. The report will detail any problems encountered or departures from stated protocols and their potential impacts on the program.

Composite samples for potential chemical analyses will be prepared from samples collected from all five sites in 2004 and three sites in 2005 (Deer Island Flats, Outfall Site, and Cape Cod Bay). Three groups of 5 individual fish each will be pooled from the 15 collected to create three pooled samples per site. Two tissue types (fillet, liver) are to be composited. This will result in 30-pooled samples (3 pools x 5 sites x 2 tissue types) in 2004 and 18 pooled samples in 2005. The same fish will be composited for both liver and fillet chemistry to ensure comparability between tissue types. The chemical analyses that may be performed on sample and tissue types are indicated in Table 3 and are described in detail in Version 0 of the Fish and Shellfish Monitoring CW/QAPP (Lefkovitz *et al*, 2002).

Table 3. Summary of Chemistry Parameters to be Measured by Organism.

Sample Type	Number of Samples	Metals (other than Hg and Pb)	Hg	Pb	PCBs	PAHs	Pesticides	Lipids
Flounder Meat	9[1]		*		*		*	*
Flounder Liver	9[1]	*	*	*	*	*	*	*

[1] 15 samples during the 2004 survey.

7.3.2 Flounder Histological Analysis (Task 25)

The fifty flounder from each of the 5 sampling sites will be analyzed for the suite of histological parameters. One 5 μm thick section from each of three transversely cut portions of livers from each flounder collected during each survey will be examined histologically. A total of 250 slides each containing 3 liver sections, will be prepared and examined each year (2004 and 2005). Lesions to be scored include vacuolation (tubular hydropic, centrotubular, focal hydropic), macrophage aggregation, biliary duct proliferation, neoplasia, and apoptotic lesions.

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

7.4 Monitoring Parameters, Collection Frequency and Sample Collection Requirements

Table 3 summarizes the primary chemical parameters that may be measured for each flounder sample type (Task 24).

Table 4 summarizes the number of organisms and the types of analyses that will be conducted on samples collected from each station as well as the sample container and preservation requirements.

Table 4. Monitoring Parameters, Collection Frequency, Sample Containers, and Preservation Requirements.

Organism	Parameter	Numbers of Sampling Units Total[a]/Sample[b]	Container	Shipboard or Laboratory Processing/Preservation	Holding Time from Collection
Winter flounder	Chemistry (archival) - liver - edible tissue	 15/3 15/3	Clean, labeled jar; Organics: Glass Inorganics: pre-cleaned polystyrene	Laboratory: Freeze, if not processed immediately	One year (If not frozen - Hg: 28-d; inorganics: 6-mo)
	Histology	50/50	Clean, labeled jar	Laboratory, Shipboard: 10% neutral buffered formalin	NA
	Age (scales)	50/50	Age envelope	Laboratory, Shipboard: Clean mucous from sampling area of fish before taking scales	NA
	Visual	50/50	N/A	Laboratory, Shipboard: Describe qualitatively	NA
	Biometrics - weight - standard length - total length - sex	50/50	N/A	Laboratory, Shipboard: Describe quantitatively	NA

a = total individual specimens collected per station.
b = total pooled (composite) samples to be analyzed per station.

7.5 Whale Observations

During every field activity under this Project conducted between January 1 to May 31 and December 1 to December 31 each field year, and during all Nearfield water column surveys carried out under Task 9, whale observations will be conducted using trained dedicated observers. Therefore, whale observations will be collected during the Task 21 flounder survey each year, mentioned in the survey summary, and the results detailed in the survey report.

8.0 PROJECT FISCAL INFORMATION

This project is being carried out under the Harbor and Outfall Monitoring contract (Contract No. S366) between MWRA and Battelle.

9.0 SCHEDULE OF ACTIVITIES AND DELIVERABLES

The schedule of activities and deliverables for this project is tied to survey activities.

Table 5 provides the 2004-2005 planned schedule for all survey plans, survey cruises, survey reports, and data reports required for Tasks 21 and 25. The deliverables are survey plans, survey reports, and data reports for each survey per year. Data synthesis will occur under Task 33. Draft and final Annual Fish and Shellfish Reports will be prepared for each year. The due dates for the data reports are shown in Table 5.

Table 5. Schedule of Deliverables.

Task	Deliverable	Due Date
Flounder Survey (Task 21)	Survey Plan Survey Cruise Survey E-mail Survey Report	March of each field year April of each field year 2 days after each survey May of each field year
Flounder Histology Analysis (Task 25)	Histology Data Report	August 15 (temporary data report for liver disease incidence at Outfall Site – 60 days after survey)
Fish and Shellfish Annual Report (Task 33)	Report Outline Synthesis Report	January of the following year February of the following year

[1] Whichever date is later.

10.0 PROJECT ORGANIZATION AND RESPONSIBILITIES

The Fish and Shellfish Monitoring tasks will be accomplished through the coordinated efforts of several organizations. Figure 3 presents the Project Management structure and the major tasks necessary to complete the scope of work. Each element of the tasks has been assigned a separate subaccount with budget and milestones, and these accounts will be used to track costs against progress. Battelle's Project Management Plan describes the management policies that will be applied to all HOM 4 activities (Battelle, 2002).

Dr. Andrea Rex is the Director of the MWRA Environmental Quality Department. Dr. Michael Mickelson is the MWRA Project Manager. Mr. Maury Hall is the MWRA Project Area Manager for all Fish and Shellfish activities. Mr. Ken Keay is the Deputy Project Manager and serves as backup to both Dr. Mickelson and Mr. Hall. They will be informed of all matters pertaining to work described in this CW/QAPP. Ms. Wendy Leo is the MWRA EM&MS Database Manager and Quality Assurance Officer.

Ms. Ellen Baptiste-Carpenter is the Battelle Project Manager. She is responsible for ensuring that products and services are delivered in a timely and cost-effective manner that meets MWRA's expectation, and for the overall performance of this project. Dr. Carlton Hunt is the Battelle Technical Director and is responsible for ensuring that data collection and interpretation are scientifically defensible, and for responding to technical challenges as they arise. Ms. Jeanine Boyle is the Battelle Deputy Project Manager. The Battelle Quality Assurance Officer for the project is Ms. Rosanna Buhl. For this task, Ms. Buhl is responsible for reviewing data reports and QA Statements submitted by subcontractors for quality completeness and adherence to the CW/QAPP. She is also responsible for reviewing the data and synthesis reports for accuracy and completeness. Mr. Chris Gagnon is the Battelle Field Manager, responsible for the overall field program. Ms. Deirdre Dahlen, Battelle's Laboratory Manager, is responsible for overseeing all laboratory activities in the contract. Ms. Ellie Baptiste-Carpenter is also Battelle's Database Manager for this project. The key contacts at each of the supporting laboratories are shown in Figure 3. Addresses, telephone (and fax) numbers, and Internet addresses, as well as specific project roles and responsibilities, are presented in the HOM 4 Program Management Plan.

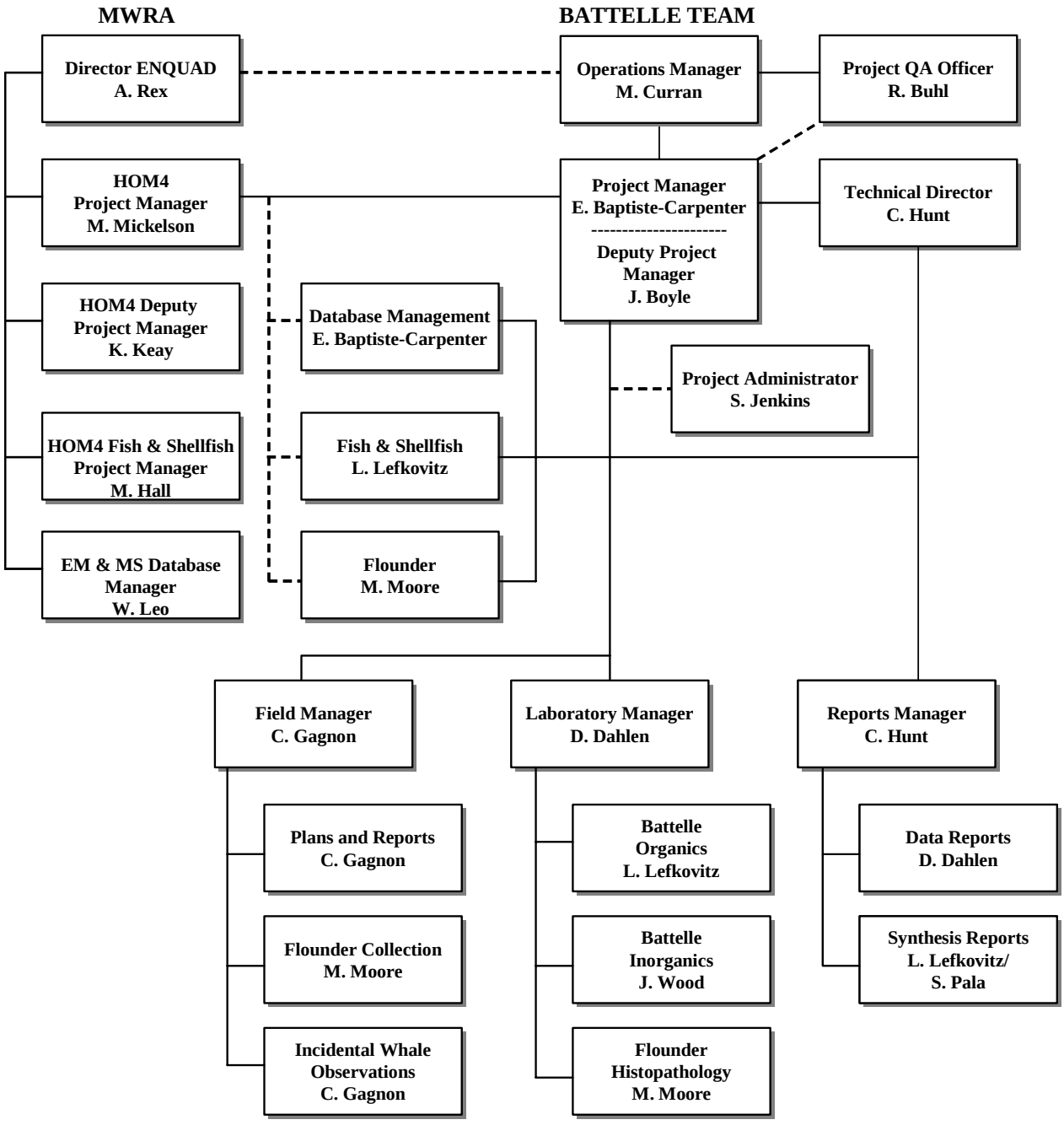

Figure 3. Organizational Chart.

Ms. Lisa Lefkovitz is the Battelle Senior Scientist responsible for the conduct of the fish and shellfish monitoring tasks described in this CW/QAPP.

Dr. Michael Moore (WHOI) is the Senior Scientist for the Flounder Survey. Histological slides will be prepared at Experimental Pathology Laboratories under subcontract to WHOI. Dr. Moore will examine the histological slides, analyze and reduce the histological data, and add them to the ongoing temporal and spatial data summaries. Dr. Robert Hillman (Battelle) will serve as the back-up to Dr. Moore.

11.0 DATA QUALITY REQUIREMENTS AND ASSESSMENTS

To ensure that all data generated during the conduct of surveys, analyses, and reporting are of the highest quality, data will be examined in terms precision, accuracy, completeness, comparability, and representativeness. These terms are defined in the HOM4 Quality Management Plan (Battelle, 2002). The application of these measures of data quality is described below.

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

11.1 Navigational Data

11.1.1 Precision and Accuracy

All dGPS units have a design positional accuracy of 15 m. Based on manufacturer specifications or Battelle's experience, precision and accuracy objectives for navigation and station depth are presented in Table 6. Section 12 provides details on relevant sampling procedures to ensure data quality, and Section 14 discusses instrument calibration methods.

Table 6. Accuracy and Precision of Instrument Sensors.

Sensor	Units	Range	Accuracy	Precision
dGPS Navigation	degree	Coastal	$9x10^{-5}$ deg (10 m)	$1.8x10^{-5}$ deg (2 m)
Loran-C Navigation	nautical mile	Coastal	0.1-0.25 nm	18 – 90 m

11.1.2 Completeness

For most flounder surveys, the fishing vessel's navigation system will be used. For the flounder surveys, navigation data will be 100% complete. The initial and final coordinates of each flounder trawl will be hand recorded on field logsheets.

11.1.3 Comparability

Latitude/longitude positions will be comparable to positions obtained by previous MWRA monitoring activities as well as by other researchers that have used or are using differentiated GPS at these stations. The station locations are targets and sampling for flounder will be conducted within 300 m of the targets but will ultimately be based on the availability of individuals.

11.1.4 Representativeness

The representativeness of the sampling program design is detailed in the Outfall Monitoring Plan (MWRA 1997) and defined by the results collected since 1992. Representativeness will also be ensured by proper handling, storage, and analysis of samples so that the materials analyzed reflect the collected material.

11.2 Flounder Collection (Task 21)

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

11.2.1 Accuracy

Traditional measures of accuracy do not apply to fish collection procedures. To ensure that specimens are accurately 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 "Normark" fish scale is 50 g. The accuracy of the fish measuring board is 0.1 cm.

11.2.2 Precision

The precision of fish length and weight measurements will be monitored through the re-measurement of at least 10% of the specimens collected at each sampling site. If agreement between the length or weight measurements is within 1 cm or 5 g, respectively, measurements will continue. If measurements or weights differ by more than the above-stated criteria, the cause will be identified and all specimens measured since the last acceptable precision measurement will be re-measured or re-weighed. The precision of the weight data transcription for flounder will be enhanced by using a scale (OHAUS® dial scale) with a maximum reading pointer (MRP) that retains the weight reading of the fish until another fish is put on the scale.

11.2.3 Completeness

The objective is to obtain 50 sexually mature specimens from each sampling site. Otter-trawl tows will be conducted until at least 50 specimens are collected at each sampling site. Sampling will be 100% complete when this is accomplished. In the event of sample loss or equipment malfunction, the Chief Scientist will determine the need for appropriate corrective action (e.g., re-sampling using a different otter trawl). The corrective action taken by the Chief Scientist will be recorded in the survey records. In the event of inadequate numbers of fish, three hours of bottom time will be the maximum effort expended at any one station. In the event of 3 hours bottom time failing to yield 50 fish, additional fish from other stations may, at the discretion of the MWRA, be sampled to generate a total of 250 fish for the survey.

11.2.4 Comparability

Winter flounder have been routinely used by other researchers working in Massachusetts Bay and similar environments to determine health and tissue burden of contaminants. Collection of winter flounder that are sexually mature will allow a comparison of the health of adult specimens at each sampling site. The

methods of collection and visual analyses are comparable to methods used by Dr. Michael Moore for previous studies on winter flounder from these sampling sites. Otter trawls have been used by the National Oceanic and Atmospheric Administration (NOAA) National Marine Fisheries Service (NMFS) and the Massachusetts Division of Marine Fisheries since the 1960s and 1970s, respectively, as a method to sample finfish. All otter-trawl tows will be conducted for approximately 30 minutes at a speed of 1.5 to 2 kt. The sampling design of this survey is comparable to the design of previous surveys.

11.2.5 Representativeness

Representativeness is primarily addressed through sample design. 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 Flounder Histological Analysis (Task 25)

11.3.1 Accuracy

Traditional measures of accuracy do not apply to flounder histology analyses. Flounder scales and otoliths will be read by National Marine Fisheries Service (NMFS) scientists that are experienced in aging winter flounder.

11.3.2 Precision

A percentage of the scales will be reread to verify age determinations. Histological observations of tissue abnormalities and scores assigned to these abnormalities are somewhat subjective based on the opinion of the pathologist reading the slides. Precision and accuracy of the measurements are therefore difficult to define quantitatively. However, an intercomparability exercise carried out in 1993 documented that 2 trained pathologists looking at the same material, identified roughly equivalent frequencies and severities of lesions (Hillman *et al.* 1994). Another comparability study was performed by Moore *et al.* (1993) in which a blind re-evaluation of 1989 slides was performed in 1993 showing 100% agreement. These findings suggest that, although quantification of the accuracy and precision of the protocols is difficult, it is measurable and has been demonstrated to be acceptable.

11.3.3 Completeness

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

11.3.4 Comparability

The lesions scored, the method of scoring, and the data reduction and analyses will be similar to what has been done in previous years under the HOM program. Scales will be read as a courtesy by NMFS scientists that have aged winter flounder during the previous studies. Comparability of flounder liver histology data has been confirmed in a number of studies described in Section 11.3.2. Several slides will be studied with Dr. Robert Hillman to assure that observations are comparable to those made during studies conducted previously.

11.3.5 Representativeness

The program design and objectives ensure representativeness.

12.0 SAMPLING AND ANALYTICAL PROCEDURES

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

12.1 Navigation

During the flounder collection surveys, navigation data will be collected from dGPS and/or LORAN aboard the vessel used and will be hand recorded on a field logsheets.

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 2. The tows will be conducted for 15-30 minutes at a speed of 1.5 to 2 kt in a direction parallel to lobster-pot trawls in the area to avoid interaction with lobster pots. Tows will be conducted until at least 50 specimens have been collected at each sampling site. At the start and completion of each tow, the time and vessel position will be recorded by differential GPS and/or LORAN.

2. At the completion of each tow, the otter trawl will be brought on board using a mechanical winch and the contents of the trawl unloaded onto the aft deck of the vessel. It may be necessary to conduct more than one otter-trawl tow at a sampling site if the required number of specimens greater than 30 cm total length (50) is not collected during the first tow. If the required number of flounder is not collected after one 30-minute tow and three 1 hour tows at an appropriate adjacent site, collections at that site will be terminated for the survey period (though individual tows may be up to 1 hour if necessary). If the number of fish in the first hour of towing is less than five, the effort will be deferred for two to four weeks. This strategy has proven to be efficient in previous years.

3. All specimens will be sorted by species, however, only winter flounder will be retained; other species will be returned to the environment. If unusually large (> 50 cm) winter flounder are obtained, up to three (3) large individuals per site will be retained for processing. With the approval of MWRA's Project Manager, some such individuals may be analyzed under Tasks 24 and 25 as extra units.

4. Fish held for potential chemical analysis will be kept on ice and hand-delivered to Battelle Duxbury Operations. If a fish is collected and assigned a sample ID but then dies, a comment will be made on the flounder collection form (Figure 4). Data will not be collected from this individual.

12.2.2 Tissue Sample Processing

Processing will be conducted in the laboratory for the 15 fish for histology and potential tissue chemistry[3] analysis and on board the collection vessel for the 35 or 50 fish for only histology analyses.

[3] In 2005, only fish from DI, Outfall, and ECCB will be processed for potential chemical analysis.

12.2.2.1 Sample Processing for Histology Analyses

1. The fish from each site will be processed for histology analyses immediately, (this process may continue while proceeding to the next sampling site). The fish will be killed by means of a cervical section prior to processing.
2. The weight, standard length, and total length will be determined (see Figure 2 and SOP 5-175). Each flounder will be examined for external evidence of disease (fin rot and external lesions) and notes will be recorded on the flounder sampling log (Figure 4). More precise external lesion information will be recorded on the form in Appendix B, which gives examples of the different types of external lesions.
3. Scales will be collected from specimens on board the vessel. Scales will be removed after first removing any mucus, debris, and epidermis from the dorsum of the caudal peduncle by wiping in the direction of the tail with a blunt-edged table knife. Scales will be collected from the cleaned area by applying quick, firm, scraping motions in the direction of the head. Scales will be placed in the labeled age-sample envelope by inserting the knife between the liner of the sample envelope and scraping off the scales.
4. The livers will be removed and examined for visible gross abnormalities (gross liver lesion). They will be preserved in 10% neutral buffered formalin for histological analysis. Liver samples from each fish will be placed in a separate clearly labeled sample container.

12.2.2.2 Sample Processing for Potential Tissue Chemistry

Because contaminant-free conditions cannot be found on board the vessel used for flounder collection, the fish used for chemical analysis will be returned to the laboratory for organ dissection. Of the 50 flounder collected from each site for histopathological analysis, 15 fish will be designated for tissue chemical analysis. The fish will be held on ice, and stored in separate, site-specific coolers, until they are returned to the laboratory.

The flounder tissues will be removed in the laboratory under contaminant-free conditions. Tissue processing will be conducted in Battelle's tissue laboratory. Fish will be processed for histology analysis as described in Section 12.2.2.1, then, using a pre-cleaned (i.e., rinsed with 10% HCL, Milli-Q (18 megohm) water, acetone, DCM, and hexane) stainless steel knife, the fillets (muscle) will be removed from the flounder and the skin will be removed from the fillet. Composites will be composed of equal weights of the

Station #:_____
Station ID:_____
Collection Date/Time:_____

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			Chain of Custody:	Check applicable sample type	Scale: _____ Histology: _____	Chem Liv: ___ Fil:___	Relnq___/__/__ Rec'd___/__/__		Relnq___/__/__ Rec'd___/__/__			External Lesions (0 to 4)
Event	ID	STN	Composite #	Total Length mm	Standard Length mm	Weight. (g)	Sex	Age	Liv. col.*	Fin (0 to 4)	Gross Liver Lesion (0 to 4)	
F00	1001	DIF										
F00	1002	DIF										
F00	1003	DIF										
F00	1004	DIF										
F00	1005	DIF										
F00	1006	DIF										
F00	1007	DIF										
F00	1008	DIF										
F00	1009	DIF										
F00	1010	DIF										
F00	1011	DIF										
F00	1012	DIF										
F00	1013	DIF										
F00	1014	DIF										
F00	1015	DIF										

Scientist:_____ Date Collected:__/__/__ QA Officer:_____ Page __ of __

*Y: yellow, YB: Y Brown, B: Brown, DB: Dark B. Date:__/__/__

Figure 4. Sample Collection Log — Winter Flounder.

homogenates of 5 individual fish that are prepared using approximately equal masses of top and bottom tissue. Homogenization will be performed using a stainless steel TEKMAR® tissuemizer. Each composite will be placed in a sample container clearly identified with the unique sample identifier.

Livers from the 15 fish selected for chemical analyses will be removed using a titanium or ceramic knife and will also be analyzed for chemical parameters. Following the processing for histology analysis, the livers will be individually homogenized by finely chopping with the titanium or ceramic knife and divided into three separate composites to correspond to the composites made for the fillets. This is done to ensure comparability between fillet and liver chemical analyses. Each composite will be placed in a sample container clearly identified with the unique sample identifier. Note: The liver composite samples will contain approximately equal masses (5 grams) from each of the livers being used in the composite. For fish with extremely small livers (< 5g wet weight), all available liver tissue will be used from such fish.

Following processing of livers for histology analysis, the homogenized tissue and liver samples will be frozen and stored. Any remaining tissue from each specimen will be archived frozen should additional analysis be required under Task 24.13.

12.3 Flounder Histological Analysis (Task 25)

Livers of 50 flounder from each site will be processed for histological analysis by Experimental Pathology Laboratories in Herndon, VA as described below. The age of each flounder will be determined by NMFS scientists through analysis of growth rings (annuli) on the scales removed during the conduct of the Flounder Collection (Task 21) as described in Section 12.2.2.

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

Each slide will be examined by Dr. Moore at WHOI 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
- Apoptotic lesions (i.e. balloon cells)

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

13.0 SAMPLE CUSTODY

A unique nine character *Sample ID,* will identify samples collected in the field. The *Sample ID* will identify the sample collected (i.e. a single flounder). The five character *Event ID* will be unique to each

survey, such as "FF021", with "FF" indicating that it is a Flounder survey, "02" indicating the survey year, and "1" signifying the first survey of the year. For individual flounder, the *Sample ID* will consist of the *Event ID*, the Station ID[4], and a three digit sequential number (001-050 for flounder). The *Composite ID* is a four place alphanumeric laboratory ID (XX00) that also serves as the *Bottle ID*. Unique *Bottle ID*s are assigned to edible tissue and liver tissue from each fish.

13.1 Custody of Electronic Data

13.1.1 Navigation Data

Custody of any electronic navigation data will be the responsibility of the Chief Scientist during the field activity. For the flounder surveys, survey data, including survey ID, date, start and stop time, and vessel position at start and completion of each sampling event, will be hand-recorded in the survey logbook. The Battelle Field Manager must receive a complete copy of the survey log for each survey.

13.1.2 Laboratory Data

Battelle may produce electronic data under this task. At Battelle, the electronic files for chemical data will remain in the custody of the analysts until all analyses are completed and data have gone through the Battelle Quality Assurance Unit. The electronic data will be transferred the HOM4 Database Manager for entry into the MWRA database. If the chemical analysis option is exercised, custody of laboratory data will be monitored according to Version 0 of the CW/QAPP for Fish and Shellfish Monitoring: 2002 – 2005 (Lefkovitz *et al*., 2002).

13.2 Flounder Samples

During field collection, custody forms will be completed. Manual entries will be recorded in indelible ink in the data section of the chain-of-custody. Based on the types of samples that will be collected, chain-of-custody forms will be generated for each sample type to track samples from collection to laboratory. Figure 5 shows an example of a chain-of-custody form that will be used.

The samples will remain in the custody of the Chief Scientist (designated for each survey) while in the field. Custody forms will accompany the samples when transferred from the field to the laboratory. All samples will be distributed to the appropriate laboratory personnel by hand or by Federal Express. When samples arrive at the laboratory, custody will be relinquished to the Laboratory Custodian. Upon receipt of the samples, the laboratory Sample Custodian will examine the samples, verify that sample-specific information recorded on the custody forms is accurate and that the sample integrity is uncompromised, log the samples into any laboratory tracking system, complete the custody forms, and sign the custody form so that transfer of custody of the samples is complete. Completed custody forms must be faxed to the Battelle custodian within 24 hours of sample receipt. Any discrepancies between sample labels and transmittal forms, and unusual events or deviations from the project CW\QAPP will be documented in detail on the custody form and the Senior Scientist and Laboratory Manager notified. The original custody forms will be submitted to the Battelle MWRA Laboratory Manager with the data and maintained in the MWRA project files. Due to the complexity of the field IDs, unique laboratory specific sample IDs may be assigned to individual composite samples during sample Log-in.

When samples are composited, a compositing form will be completed (see Figure 6).

[4] Flounder station IDs are: 1 (DI); 2 (Nantasket); 3 (BS); 4 (Outfall); 5 (ECCB)

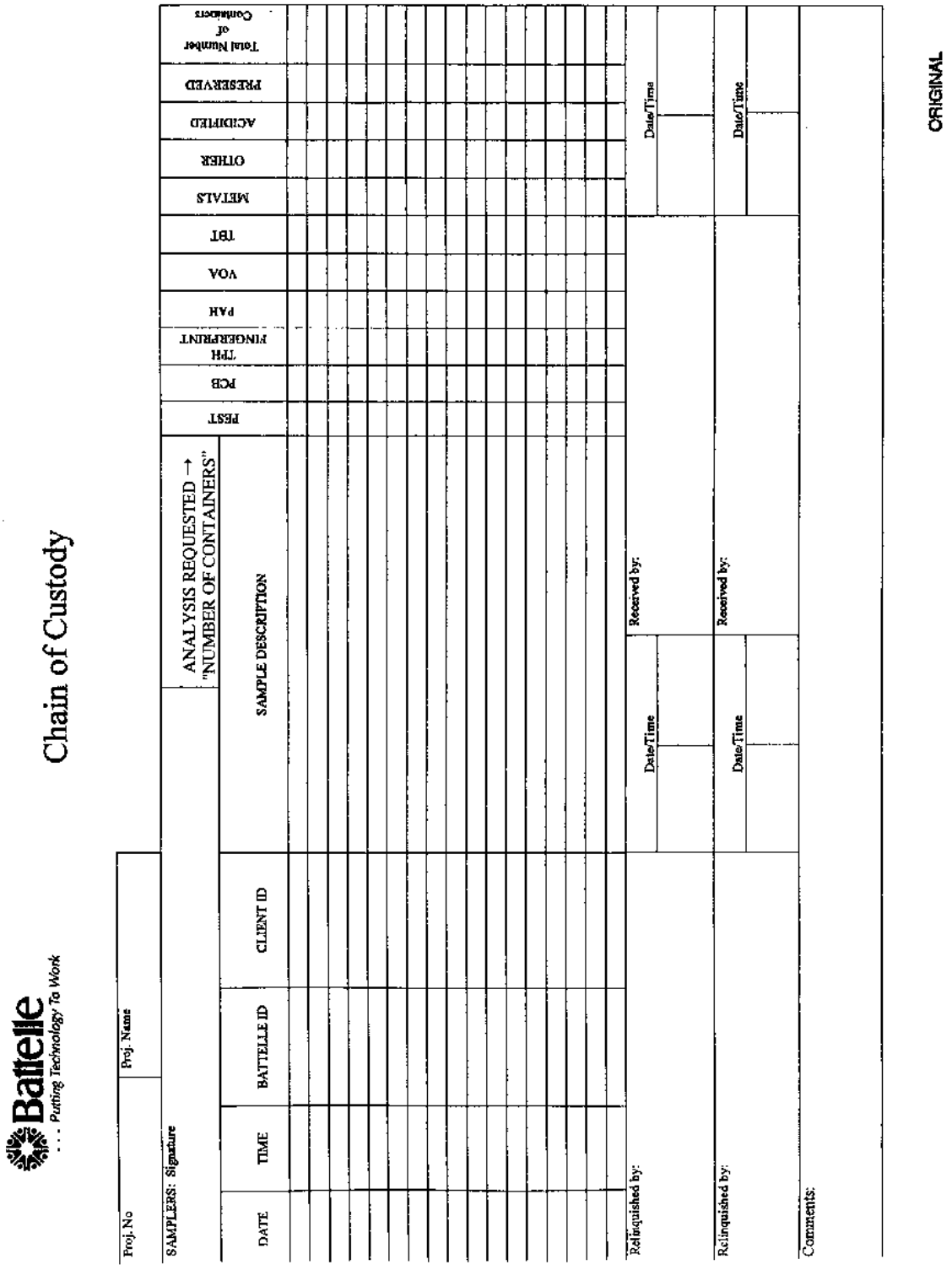
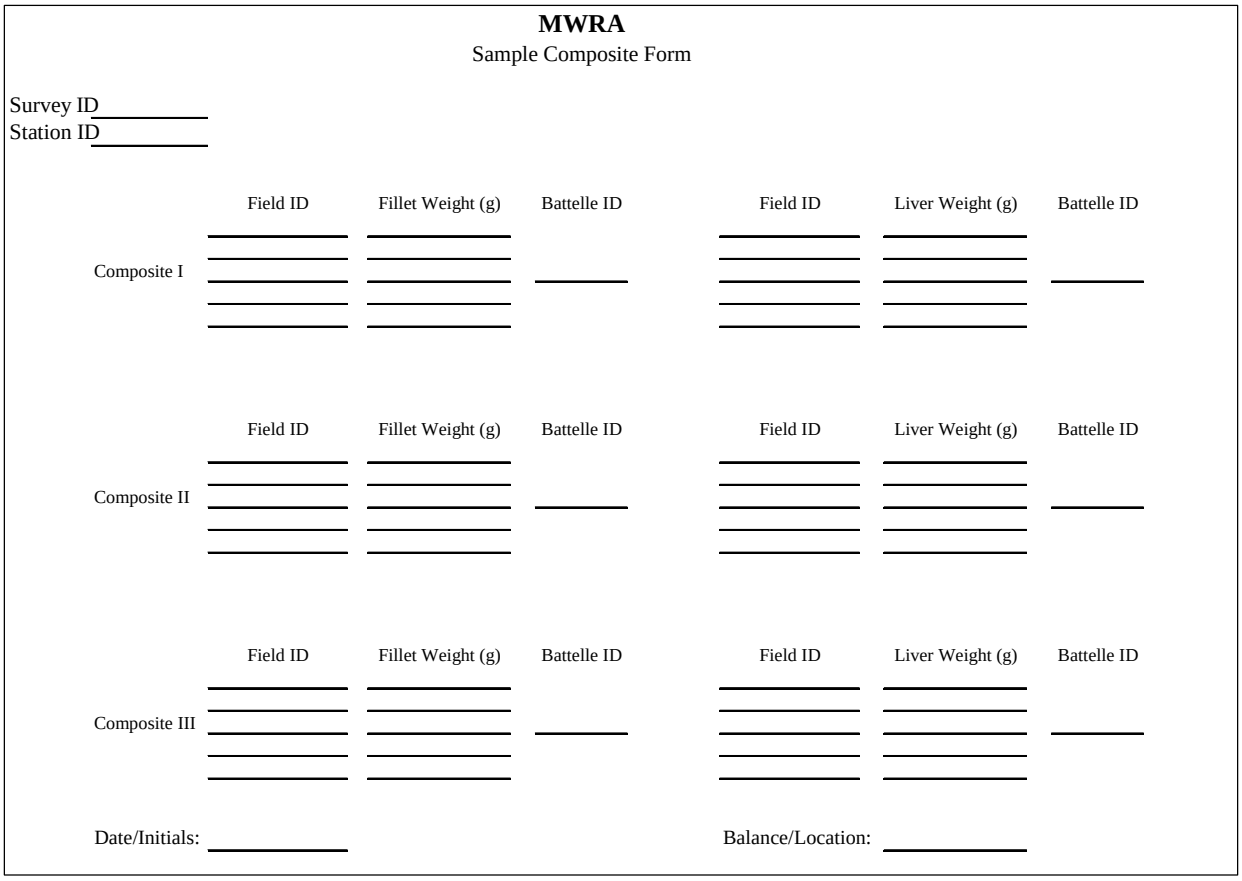


Figure 5. Field Chain of Custody Record.



MWRA

Sample Composite Form

Survey ID _____
Station ID _____

	Field ID	Fillet Weight (g)	Battelle ID	Field ID	Liver Weight (g)	Battelle ID
Composite I						
Composite II						
Composite III						

Date/Initials: _____ Balance/Location: _____

Figure 6. Example Sample Compositing Log – Flounder.

13.3 Histology Samples

The laboratory custodian of samples for histologic analyses will be Dr. Michael Moore, of WHOI. He will be responsible for shipping the samples to be histologically processed to Experimental Pathology Laboratories in Herndon, VA, where chain-of-custody forms will be signed by the receiving histology technician Keith Rogers. The tissue slices will be embedded in the same tissue cassettes labeled at the time of collection. Sample numbers will be copied from the cassettes to the slides at the time of sectioning the embedded blocks. The blocks and slides will be returned to Dr. Moore, chain-of-custody forms signed again, and all histology material thereafter will be archived at WHOI.

14.0 CALIBRATION PROCEDURES AND PREVENTIVE MAINTENANCE

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

14.1 Navigation Equipment

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

14.2 Field Equipment

Equipment will be monitored and/or calibrated according to the following methods:

- Measuring boards used to measure flounder will be wiped dry after measuring every 10th specimen or as needed and will be rinsed after sampling has been completed at each sampling site.
- The OHAUS® dial scale, Model No.8014 MA, will be dried after weighing every 10th fish or as soon as water starts to accumulate 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.

14.3 Histological 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.

15.0 DATA DOCUMENTATION, REDUCTION, AND REPORTING

15.1 Documentation

Documentation will include sample collection logs, chain-of-custody forms, and laboratory records. Sample collection information will be recorded on standard forms that, at a minimum, should include sample location, time and date, sampler's identification, and sample ID number. An example of the sample collection log for flounder is given in Section 12.0 (Figure 4). Chain-of-custody records are discussed in Section 13.0.

All data will be initially recorded manually into bound laboratory notebooks or onto established data forms. All notes will be written in black ink. Corrections to hand-entered data will be initialed, dated, and justified. Completed data forms or other types of hand-entered data will be signed and dated by the individual entering the data. Direct-entry and electronic data entries will indicate the person collecting or entering the data. 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 (below). In addition to these documentation procedures, station logs or copies thereof and associated field custody forms will be kept in a survey notebook for each survey. These notebooks will be stored under the supervision of Ms. Jeanine Boyle. Laboratory tracking forms will be kept with the analytical data packages for each batch.

15.2 Data Reduction

Data reduction involves the process of converting raw numbers into data that have direct physical, biological, or chemical meaning and can be compared statistically. The data discussed in this section are those data that require some manipulation before being submitted to Battelle data management for entry into the EM and MS database.

15.2.1 Navigation Data

Navigation data are recorded to 7 decimal places. No data reduction is performed. During surveys where NavSam© is not used, all sample IDs and sample collection information will be recorded by hand and transferred to an electronic format (i.e. MS Excel) with date, time, and concurrent dGPS/LORAN vessel-position data.

15.2.2 Histopathological and Morphological Data

Flounder Field Data – The Catch Per Unit Effort (CPUE – fish caught per minute of bottom time) will be calculated at each flounder sampling station. CPUE is calculated as the total number of flounder caught per unit of bottom trawl time. The gross external condition ("External Lesions") of each flounder is rated on a scale of 0 to 4. The severity of fin rot and gross liver lesions are scored from 0 to 4, where: 0 = absent; 1 = minor; 2 = moderate; 3 = severe; and 4 = extreme.

Flounder Liver Histology – From the prepared liver sections, the severity of each flounder liver 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 severity index will then be calculated as a mean of scores from three slides. Data resulting from the assignment of scores to the various lesions will be transferred in electronic format to database personnel.

15.3 Data Entry, Loading, and Reporting

15.3.1 Data Loading Applications

The data reporting for analytical and experimental data begins with the Battelle Data Management Team who will populate a loading application for each laboratory. The loading applications are populated with the Sample_ID numbers and analysis protocols extracted from the Access database containing data from field activities that is delivered to the data manager at the conclusion of each survey. A separate loading application will be prepared for each data deliverable.

15.3.2 Population of Loading Applications by Battelle

Analytical laboratories with existing data processing capabilities (Battelle Duxbury and Battelle Sequim) will provide their laboratory's final computer-generated data spreadsheets to Battelle if the analytical chemistry option is exercised. The data from field activities will be delivered to the data manager as an Access database. The Battelle data management team will use a loading application to run the necessary quality control checks and load the data provided into the ORACLE database. Battelle uses generic loading applications that are designed to process large analytical datasets that are received in spreadsheet form and converts them into the correct format for entry into the ORACLE database. Each laboratory will have to meet its own internal laboratory format for the data to load successfully.

15.3.3 Population of Loading Applications by Other Laboratories

When data contributors (Woods Hole Oceanographic Institution) open the database within the appropriate loading application, they will be presented with a form that already contains the Sample_ID numbers and a parameter list for the required data submittal. The laboratory will enter the results of the analyses and other supporting information such as data qualifier codes. All entries will be constrained by the rules of EM&MS. Errors will be caught on entry and fixed by the data contributor. Primary keys will be in place so duplication cannot occur. Entry applications will be developed for each analytical laboratory. Laboratory staff receive one day of training on the application prior to analysis of the lab's first set of samples. When data entry is complete, the database will be sent back to Battelle.

The loading application will provide the laboratory with several function buttons. These include hard copy report, quality control checks, exception report, and analysis summary. The hardcopy report function button will allow the laboratory to create a hardcopy report to check for entry errors and to submit a final hardcopy report to Battelle along with the electronic data deliverable. The quality control checks will be comprised of the applicable sections of EM&MS check script and will also perform checks for outliers. This report will provide the data contributor a chance to confirm the reasonableness of the data prior to its submission to Battelle. The exception report will check the data that was expected against the results that were loaded. The data contributor must account for any entries in the exception report. The analysis report will produce a report of the number of analyses by analyte. A copy of this report will be included with the data deliverable and with the invoice for the analyses.

Within the loading application, the data entered by the laboratory will be translated into the correct codes and inserted into database tables with the same structure as the matching EM&MS table. Table 7 shows the morphological parameters and database codes. Histopathological parameters and database codes for this task are shown in Table 8. Table 9 describes the database codes to be used by the laboratories. The laboratories will have the ability to add additional codes to describe their results but the new codes will be highlighted in the exception report. Battelle will notify MWRA concerning the new qualifier and will adjust the code table in the application to agree with any changes to the EM&MS code_list table. MWRA is responsible for maintaining the code list for the EM&MS.

Table 7. Morphological Parameters and Database Codes for Fish and Shellfish Monitoring.

SPECIES	PARAM_CODE	DESCR	UNIT_CODE	METH_CODE
PSEUDOPLEURONECTES AMERICANUS	AGE	Chronological age of specimen	y	SCALE
PSEUDOPLEURONECTES AMERICANUS	SEX	Gender		VISUAL
PSEUDOPLEURONECTES AMERICANUS	STAN_LEN	Standard length of a fish. From upper jaw tip to posterior end of the hypural bone.	mm	SLM
PSEUDOPLEURONECTES AMERICANUS	TOTAL_LEN	Total Length	mm	TLM
PSEUDOPLEURONECTES AMERICANUS	WEIGHT	Wet Weight of Organism	g	PWEIGHT

Table 8. Histopathological Parameters and Database Codes for Fish and Shellfish Monitoring.

SPEC_CODE	DESCR	FRACTION_CODE	PARAM_CODE	DESCR
8857041504	PSEUDOPLEURONECTES AMERICANUS	LIVER_SECTION	BALLOONS	Apoptopic lesion prevalence, rated on a scale from 0-4.
8857041504	PSEUDOPLEURONECTES AMERICANUS	LIVER_SECTION	BIL_PROLIF	Biliary proliferation
8857041504	PSEUDOPLEURONECTES AMERICANUS	LIVER_SECTION	CENTRO_HV	Centrotubular hydropic vacuolation
8857041504	PSEUDOPLEURONECTES AMERICANUS	LIVER_SECTION	FOCAL_HV	Focal hydropic vacuolation
8857041504	PSEUDOPLEURONECTES AMERICANUS	LIVER_SECTION	MACROPHAGE	Macrophage aggregation, rated on a scale from 0-4.
8857041504	PSEUDOPLEURONECTES AMERICANUS	LIVER_SECTION	NEOPLASM	Neoplasia prevalence, rated on a scale from 0-4.
8857041504	PSEUDOPLEURONECTES AMERICANUS	LIVER_SECTION	TUBULAR_HV	Tubular hydropic vacuolation
8857041504	PSEUDOPLEURONECTES AMERICANUS	WHOLE_BODY	FIN_ROT	Fin rot score
8857041504	PSEUDOPLEURONECTES AMERICANUS	WHOLE_BODY	GROSS_LIV_LESIONS	Gross lesions visible on whole flounder liver
8857041504	PSEUDOPLEURONECTES AMERICANUS	WHOLE_BODY	EXT_LESIONS	Gross external lesions on flounder body
8857041504	PSEUDOPLEURONECTES AMERICANUS	WHOLE_BODY	LIVER_COL	Liver color

Table 9. Database Codes For Fish and Shellfish Monitoring.

FIELD NAME	CODE	DESCRIPTION
ANAL_LAB_ID	BOS	Battelle Ocean Sciences
ANAL_LAB_ID	WHO4	Woods Hole Oceanographic-M. Moore
FRACTION_CODE	FILLET	Fillet of fish (edible tissue)
FRACTION_CODE	INDIVIDUAL	Measurement was made on an individual animal
FRACTION_CODE	LIVER	Analysis done on liver only
FRACTION_CODE	LIVER_SECTION	Analysis done on section of liver
FRACTION_CODE	WHOLE_BODY	Entire body or part of body described at PARAM_CODE level
GEAR_CODE	OTT	Otter trawl tow
INSTR_CODE	BAL	Balance
MATRIX_CODE	8857041504	Pseudopleuronectes americanus
MATRIX_CODE	8857041504_C	Composite of Pseudopleuronectes americanus
METH_CODE	FSF98	Method for pathology parameters described in Fish and Shellfish CW/QAPP, 1998: ENQUAD MS-49
METH_CODE	PWEIGHT	Flounder wt measurement mentioned in CW/QAPP for Fish and Shellfish Monitoring Sec 11.2.ENSR 1997
METH_CODE	SCALE	Aging by scales
METH_CODE	SLM	Standard fish length, from tip of head to base of caudal peduncle.
METH_CODE	TLM	Total length measurement using fish measuring board.
METH_CODE	VISUAL	Visual inspection mentioned in CW/QAPP for Fish and Shellfish Monitoring Sec 11.2/11.3.ENSR 1997
QC_CODE	QC	Qc sample
QC_CODE	SAMP	Normal sample
SPEC_CODE	8857041504	Pseudopleuronectes americanus
UNIT_CODE	g	grams
UNIT_CODE	mm	millimeters
UNIT_CODE	PCT	PERCENT
UNIT_CODE	y	years
VALUE_CODE	0	Absent
VALUE_CODE	1	Minor or present or pre-spawning
VALUE_CODE	2	Moderate or ripe or mature; severe concerning gross score.
VALUE_CODE	3	Severe or running eggs or undeveloped.
VALUE_CODE	4	Extreme or post-spawning
VALUE_CODE	B	Brown
VALUE_CODE	DB	Dark brown
VALUE_CODE	F	female
VALUE_CODE	M	male
VALUE_CODE	Y	Yellow
VALUE_CODE	YB	Yellow brown
VAL_QUAL	A	Value above maximum detection limit, e.g. too numerous to count or beyond range of instrument
VAL_QUAL	a	Not detected - value reported as negative or null
VAL_QUAL	aq	Not detected - value reported as negative or null. May be invalid, under investigation (Do not use).
VAL_QUAL	as	Not detected - value reported as negative or null, and not fit for use
VAL_QUAL	e	Results not reported, value given is NULL. Explanation in COMMENTS field
VAL_QUAL	eq	Not reported, may be invalid, under investigation (Do not use).
VAL_QUAL	q	Possibly suspect/invalid and not fit for use. Investigation pending.
VAL_QUAL	s	Suspect/Invalid. Not fit for use.
VAL_QUAL	w	This datum should be used with caution, see comment field

15.3.4 Loading Analytical and Experimental Data into the Harbor and Outfall Studies Database

Data submissions from the laboratory will consist of final electronic spreadsheets or final loading applications as discussed above. The submissions will be logged in upon receipt and a copy of the login will be maintained on file under the login id. Data will be loaded into a temporary table by striking a button on the application. A transfer script will copy the data into the proper table in Battelle's copy of the EM&MS. Data from the laboratories will receive a quality assurance review by Battelle after the data have been synthesized into a data report. Any issues will be corrected in the database and the script output will be supplied to MWRA with the export of the database. The MWRA check script will be run on the database prior to export. Any issues will be sent to the Battelle Data Manager via email. Any irresolvable issues in the database as a result of quality control checks (for example, stations more than specified distance from target) will also be submitted to MWRA with the data export. Processing of data and development of data reports are defined in Battelle SOP MWRA 007-01.

Field personnel will submit the sample collection data electronically as Excel spreadsheets (see Section 15.2.1). The data will be loaded into EM&MS from Excel spreadsheets, as applicable.

15.3.4.1 Loading Composite Sample Information

Flounder homogenates will be composited and tracked in the COMPOSITE table. A conceptual procedure is outlined (Figure 7) to show the logic behind the treatment of composites in the EM&MS database. In this example, flounder are collected from the field and the chemical contaminants from their tissues are analyzed. To get enough material for the analysis, and to minimize the effect of random variation among flounder, the tissues from 5 flounder are pooled. Because the concentrations of chemical contaminants are known to vary substantially among the different organs of a flounder, different organs are analyzed separately.

Each flounder collected is assigned a SAMPLE_ID in the SAMPLE table (Step 1) with a matrix code identifying them as individual flounder (8857041504) and then a BOTTLE_ID in the BOTTLE table (Step 2) denoting that each flounder is intact (WHOLE_BODY).

Even though the liver and fillet meat are dissected from the five flounder individually (two are shown in Figure 7) and then composited by fraction, we treat the processes in the database as if the five flounder were composited before the various fractions are removed. A single composite SAMPLEC_ID is created in the COMPOSITE table (Step 3) that represents all the flounder in the composite sample. There will be one record in the COMPOSITE table for each individual in the composite (five in this example, two shown in the figure).

A new record is added to the SAMPLE table with SAMPLE.SAMPLE_ID equal to COMPOSITE.SAMPLEC_ID with a MATRIX_CODE indicating that this sample is a composite flounder (8857041504_C) (Step 4). The other fields in the SAMPLE table are filled with information best describing the composite sample. For example, DEPTH would have the deepest of the five individual sample depths while DEPTH_TOP would have the most shallow.

The composite sample can now be subsampled, creating new bottles for each fraction. Since we need a bottle from which to subsample, a new bottle is created for the composite sample with a fraction code of WHOLE_BODY (Step 5). Bottles are created from this bottle for each of the fractions that will be analyzed, the fractions being recorded in the FRACTION_CODE (Step 6). The FRACTION_CODEs for flounder bottles are WHOLE_BODY, FILLET, LIVER, and LIVER_SECTION.

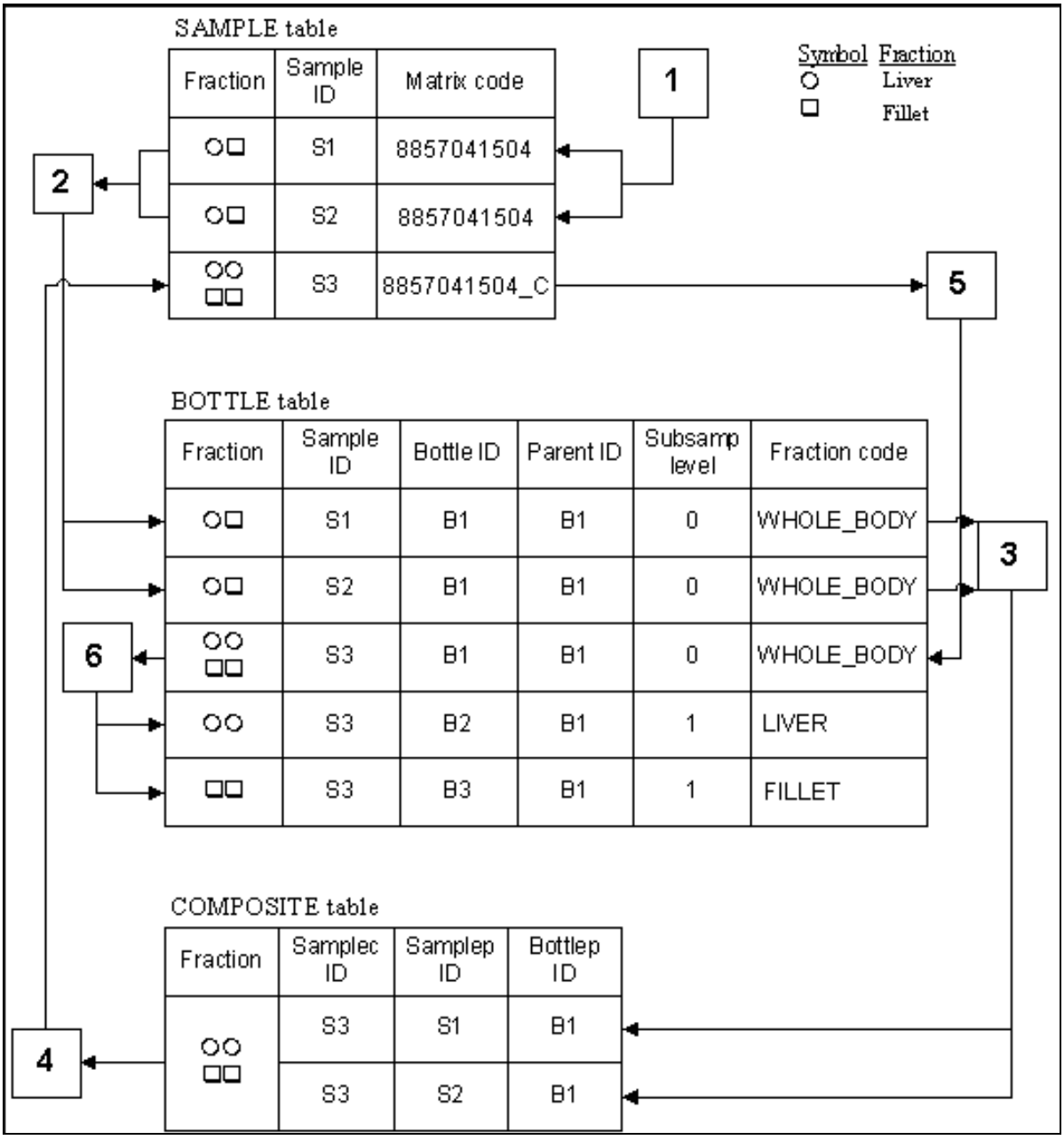


Figure 7. Conceptual Procedure for Reporting of Composite Samples.

Using this method for creating composites with fractioned sub-samples, the analytical results from different fractions from the same group of bottles will all have the same SAMPLE_ID. This facilitates queries that bring together results from different fractions coming from the same pooled bottles.

15.3.5 Data Report Quality Control Checks

Prior to data submission to MWRA, Battelle will perform a series of data report quality control checks. These include range checks of various parameters against previously accepted data for tissue chemistry (if

appropriate), morphology, and histopathology. The fish and shellfish area senior scientist, Ms. Lisa Lefkovitz, will review the results of the checks prior to submission of the data report. Table 10 presents a list of preliminary QC checks that will be performed on fish and shellfish data.

Table 10. Fish and Shellfish Data Report QC Checks.

General: For each data report a table of: • Planned analyses against actual number of analyses • Count of samples with non-detectable results • Number of null values • List of missing samples	
Type of quality Control Check	
Parameter	**Flounder** **Each tissue type**
Length	Range check against longest and shortest flounder from previously acceptable data. Flag organisms outside of this range.
Weight	Range check against longest and shortest flounder from previously acceptable data. Flag organisms outside of this range
Age	Plot Age vs. Length and Weight. Flag outliers and re-evaluate measurement.
Morphology	0-4 range check for each morphological measure. Flag organisms outside of this range.
Liver Histopathology	0-4 range check for each histopathology parameter. Flag organisms outside of this range.

15.3.6 Fish and Shellfish Threshold Evaluation

One of the requirements of the discharge permit is to test the current environmental conditions against baseline conditions to detect any noticeable changes. These thresholds are defined in the Contingency Plan (MWRA 2001). Battelle's requirement under HOM4 in regard to threshold testing is to:

- Maintain threshold, threshold_baseline, and threshold_test tables in the local copy of EM&MS
- Import new threshold and threshold_baseline tables if MWRA makes changes
- Maintain current version of threshold test scripts as provided by MWRA
- Run current version of threshold test script on newly loaded data as appropriate
- Maintain a record of all threshold runs in local copy of threshold_test table
- Report running of threshold tests in monthly progress report
- Report results of threshold tests run on data being reported in data report
- Notify MWRA as to any potential exceedances

The documentation for each threshold test is maintained by MWRA in a series of SOPs. The SOPs pertinent to the fish and shellfish task area are found in Appendix A. The threshold evaluation is performed as part of the data report.

15.3.7 Reporting Data to MWRA

The data contained in each hard copy data report will be submitted to MWRA as a database export; hard copy data reports will be prepared following Battelle SOP MWRA 007 Loading and Reporting Fish and Shellfish Data. The supporting documentation files will be included with the data submission. Data deliverables will be combined only with permission from MWRA. Station depths reported for flounder surveys are the nominal station depths maintained in the MWRA Geo_Station table.

16.0 DATA VALIDATION

The data validation procedures for this project are defined in the HOM4 Quality Management Plan. As a part of data validation, each Laboratory Manager ensures that:

- Any data that are hand-entered (*i.e.*, typed) are 100% validated by qualified personnel prior to use in calculations or entry into the database.

- All manual calculations are performed by a second staff member to verify that calculations are accurate and appropriate.

- Calculations performed by software are verified at a frequency sufficient to ensure that the formulas are correct, appropriate, and consistent, and that calculations are accurately reported. All modifications to data reduction algorithms are verified prior to submission of data to the Authority.

Electronic data loading and transfer are swift and routine; data fields and formats are defined in the CW/QAPPs. Electronic submissions are loaded to temporary files prior to incorporation into the database, and are analyzed selectively using methods such as scatter plots, univariate and multivariate analyses, and range checks to identify suspect values. Routine system back-ups are performed daily.

Once data have been generated and compiled in the laboratory, senior project scientists review data to identify and make professional judgments about any suspicious values. All suspect data are reported with a qualifier. This data may not be used in calculations or data summaries without the review and approval of a knowledgeable Senior Scientist. No data measurements are eliminated from the reported data or database and data gaps are never filled based on other existing data. If samples are lost during shipment or analysis, it is documented in the data reports to the Authority and noted in the database. The methods used to identify suspect values for each type of data are defined in Table 10.

A series of reviews by technical personnel will be implemented to ensure that the data generated for Tasks 21 and 25 meet the data quality objectives. These reviews will include the following activities.

- Data and related project records will be reviewed by laboratory personnel at the end of each working day to ensure that analytical activities are completely and adequately documented.
- The Laboratory Supervisors will be responsible for reviewing analytical results and supporting documentation.

The results of QC sample analyses will be compared to pre-established criteria as a measure of data acceptability.

The review of quality control data is a critical step in the data validation process because quality control data that are within the QAPP acceptance criteria indicate that the sample processing and analysis systems are in control. Section 11.0 discusses the quality control program for the fish and shellfish monitoring study. The quality control procedures and any applicable corrective action for out-of-control quality control data and instrumentation calibrations are described in Section 11. All quality control data that do not meet the data quality objectives will be flagged and brought to the attention of the Senior Scientist (Lisa Lefkovitz) who will determine the appropriate corrective action (e.g., re-analysis or data reported with qualifiers). As an additional data validation step, the Senior Scientist will review all data for technical reasonableness. The Battelle Field Manager will be responsible for validation of the navigation data.

17.0 PERFORMANCE AND SYSTEM AUDITS

The Battelle QA Officer for the Harbor and Outfall Monitoring Project is Ms. Rosanna Buhl. She will direct the conduct of at least one systems audit to ensure that Tasks 21 and 25 are carried out in accordance with this CW/QAPP. A systems audit will verify the implementation of the HOM4 Quality Management Plan and this CW/QAPP.

Tabular data reported in deliverables, and associated raw data generated by Battelle will be audited under the direction of the Project QA Officer. Raw data will be reviewed for completeness and proper documentation. For electronically acquired data (e.g., analytical chemistry data), Ms. Buhl will verify that computer software used to process the data has been validated. Errors noted in data audits will be communicated to analysts and corrected data verified.

Audits of the data collection procedures at subcontractor laboratories will be the responsibility of the Subcontractor. Each subcontractor is fully responsible for the QA of the data it submits. Data must be submitted in CW/QAPP-prescribed formats; no other will be acceptable. During the time work is in progress, an inspection will be conducted by the subcontractor QA Officer or their designee to evaluate the laboratory data-production process. All data must be reviewed by the subcontractor QA Officer prior to submission to the Battelle Database Manager and must be accompanied by a signed QA statement that describes the types of audits and reviews conducted and any outstanding issues that could affect data quality and a QC narrative of activities.

The Battelle QA Officer will conduct an initiation audit and, as needed, a laboratory inspection to access compliance with the Quality Management Plan and this CW/QAPP. Performance audits, in the form of SRMs, will be used to determine quantitatively the accuracy of the total measurement system or its components, will be in addition to internal performance evaluation samples and participation in external certification programs, if the analytical chemistry option is exercised.

18.0 CORRECTIVE ACTION

All technical personnel share responsibility for identifying and resolving problems encountered in the routine performance of their duties. Ms. Ellen Baptiste-Carpenter, Battelle's Project Manager, will be accountable to MWRA and to Battelle management for overall conduct of the Harbor and Outfall Monitoring Project, including the schedule, costs, and technical performance. She is responsible for identifying and resolving problems that (1) have not been addressed timely or successfully at a lower

level, (2) influence multiple components of the project, (3) necessitate changes in this CW/QAPP, or (4) require consultation with Battelle management or with MWRA.

Identification of problems and corrective action at the laboratory level (such as meeting data quality requirements) will be resolved by laboratory staff and Senior Scientist with the Laboratory Manager. Issues that affect schedule, cost, or performance of Tasks 21 and 25 will be reported to the Senior Scientist or to the Battelle Project Manager. They will be responsible for evaluating the overall impact of the problem on the project and for discussing corrective actions with the MWRA Project Manager.

Problems identified by the QA Officer will be reported and corrected as described in Section 17.0 and the Quality Management Plan (Battelle, 2001).

19.0 REPORTS

Reports that will be generated under Tasks 21 and 25 include survey plans and survey reports for each of the three surveys conducted under Task 21 and data and synthesis reports (described below).

19.1 Survey Plans, Summaries, and Reports

Each survey plan will follow the new guidelines established by EPA for use of the OSV *Anderson.* Two copies of the final survey plan will be submitted to MWRA at least two weeks prior to the survey. No draft survey plans will be prepared. Survey summaries will be delivered by e-mail to MWRA's Task Manager within two (2) business days of survey completion.

All survey reports prepared under Task 21 will contain a table which demonstrates to the MWRA that Battelle has in digital form all information specific to an individual survey (including but not limited to date, time, survey id, sample types, etc.) which is required to readily load the resulting monitoring data into MWRA's database. Survey reports will describe survey dates, vessel, personnel, methods that deviate from the CWQAPP, survey operations, results, problems encountered, corrective actions, and recommendations. The number of samples collected (versus planned) will be tabulated, and maps of the survey track lines will be provided. Observations of whales, whether noted by the whale observer or as incidental, will be described in the flounder survey report. Any unusual observations of environmental conditions, especially those with implications for the later testing of Contingency Plan thresholds, will be emphasized. Survey reports will be submitted to MWRA (two copies) within two weeks after each survey demobilization. Survey plans and reports will be produced double-sided and 3-hole punched on 20 lb paper.

19.2 Histology Data Reports (Task 25)

Histological data reports[5] (Task 25) will be a table of results and a brief discussion of any deviations from this CW/QAPP. In addition, data from Outfall Site are due 60 days after survey completion in a temporary data report that must be Quality Assured but need not meet all the requirements set forth in the General Conditions for Tasks 1-34. The temporary data report will include a calculation of the trigger parameter (i.e. liver disease incidence). The temporary data report will be discarded after the complete data report is received in August. Results of the QC checks related to flounder morphology and histopathology will be included in the final data reports. The histopathology will be discussed under the annual fish and shellfish monitoring synthesis report (Task 33.8).

[5] Data reports will be submitted as double-sided copy on 3-hole 20 lb paper. The database export will accompany the data report.

19.3 Fish and Shellfish Monitoring Annual Synthesis Report (Task 33.8)

There will be four (4) fish and shellfish synthesis reports delivered under the HOM4 contract, one for each field year (2004 and 2005). This annual report will include all data collected as part of the fish and shellfish program under Tasks 21 and 25. This report will contain an evaluation of the year's results against all relevant monitoring thresholds (Table 1), and will devote particular attention to thresholds that may have been exceeded. Such evaluation would include comparison to the baseline data, as well as whether and/or to what extent such exceedances might be attributable to MWRA discharges, and the likely environmental impact of the exceedance. The report will include an evaluation of the spatial and temporal trends in contaminants, morphology, and pathology in flounder. The conclusions from flounder will be summarized and integrated in the report, and the merits of different approaches used will be discussed.

19.3.1 Histopathology Data Analysis

For each liver lesion type, the percent prevalence will be calculated by station based on the three liver sections from each fish. The equation for percent prevalence is the number of fish showing any one lesion (in any of the three liver sections) divided by the number of fish examined and multiplied by 100. The percent prevalence of centrotubular hydropic vacuolation (CHV) is calculated as the number of fish showing any degree of CHV (in any of the three liver sections) divided by the number of fish examined and multiplied by 100. Analysis of variance will be used to compare lesions from site to site and annually from 2004-2005.

19.3.2 Analytical Chemistry Data Totals

If the analytical chemistry option is exercised, several chemistry data parameters are reported in the Fish and Shellfish Monitoring Annual Synthesis Report as totals as described in Version 0 of the Fish and Shellfish Monitoring CW/QAPP (Lefkovitz *et al*., 2002).

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Appendix A

MWRA Threshold Testing SOP for Fish and Shellfish

To: Wendy Leo, Maurice Hall, Andrea Rex
From: Joe LoBuglio, Suh Yuen Liang
Date: September 10, 2001
Revised: December 4, 2001
Subject: Calculation methods for threshold values and baselines for fish and shellfish.

There are seventeen thresholds related to chemical burdens in fish and shellfish tissues and one threshold concerning flounder liver disease. Threshold values for mercury, lead, and PCBs are based on EPA regulations while those for chlordane, DDT, dieldrin, PAHs, and liver disease are based on measurements taken during baseline years. The thresholds for chlordane, DDT, PCB, and PAH are based on the sum of concentrations of several chemicals.

Revision History:

Revision 1: Mussel bioaccumulation outfall site station is now called 'M4' exclusively.

Table 1. Summary of Threshold Values for Fish and Shellfish Thresholds.

Organism	Threshold ID	Parameter	Unit of Measure	Threshold Value		Baseline years
				Caution	Warning	
Flounder	FFFCHL	lipid-normalized chlordane	ng/g lipid	484		1993-2000*
	FFFDDT	lipid-normalized DDT	ng/g lipid	1552		1993-2000*
	FFFDIEL	lipid-normalized dieldrin	ng/g lipid	127		1993-2000*
	FFFHG	mercury	ug/g wet	0.5	0.8	N/A
	FFFPCB	PCB	ng/g wet	1000	1600	N/A
	FFLIVDIS	liver disease incidence	%	44.94		1991-2000
Lobster	FLMCHL	lipid-normalized chlordane	ng/g lipid	150		1992-2000
	FLMDDT	lipid-normalized DDT	ng/g lipid	683		1992-2000
	FLMDIEL	lipid-normalized dieldrin	ng/g lipid	322		1992-2000
	FLMHG	mercury	ug/g wet	0.5	0.8	N/A
	FLMPCB	PCB	ng/g wet	1000	1600	N/A
Mussel	FMUCHL	lipid-normalized chlordane	ng/g lipid	205		1992-2000**
	FMUDDT	lipid-normalized DDT	ng/g lipid	483		1992-2000**
	FMUDIEL	lipid-normalized dieldrin	ng/g lipid	50		1992-2000**
	FMUPAH	lipid-normalized PAH	ng/g lipid	2160		1992-2000**
	FMUHG	mercury	ug/g wet	0.5	0.8	N/A
	FMUPB	lead	ug/g wet	2	3	N/A
	FMUPCB	PCB	ng/g wet	1000	1600	N/A

* 1992 flounder data excluded because compositing scheme not compatible with other years.
** Data for 1995 not available because mussel cages could not be recovered at baseline site.
N/A Threshold not calculated using baseline data.
Data Source (Data from the EM&MS database):

Tissue Body Burdens:

- Laboratory data from the Fish and Shellfish study for the parameters shown in table 2 for the various groups are used. These data are stored in the ANALYTICAL_RESULTS table with supporting data in the BOTTLE and SAMPLE tables.

Table 2. Parameters Used for Body Burden Calculations.

Group (Group Code)	Parameter Code	Parameter Description	Parameter Abbreviation
Chlordane (CHLOR)	5103-71-9	CIS-CHLORDANE	
	MWRA25	HEPTACHLOR	
	MWRA24	HEPTACHLOREPOXIDE	
	24143-69-9	TRANS_NONACHLOR	
DDT	MWRA33	O,P-DDD	2,4'-DDD
	MWRA34	O,P-DDE	2,4'-DDE
	789-02-6	O,P-DDT	2,4'-DDT
	72-54-8	P,P-DDD	4,4'-DDD
	75-55-9	P,P-DDE	4,4'-DDE
	50-29-3	P,P-DDT	4,4'-DDT
DIELDRIN	60-57-1	DIELDRIN	
LEAD	7439-92-1	LEAD	Pb
MERCURY	7439-97-6	MERCURY	Hg
PAH (PAH_NOAA_PRE1995)	90-12-0	1-METHYLNAPHTHALENE	
	832-69-9	1-METHYLPHENANTHRENE	
	2245-38-7	2,3,5-TRIMETHYLNAPHTHALENE	
	581-42-0	2,6-DIMETHYLNAPHTHALENE	
	91-57-6	2-METHYLNAPHTHALENE	
	83-32-9	ACENAPHTHENE	
	208-96-8	ACENAPHTHYLENE	
	120-12-7	ANTHRACENE	
	56-55-3	BENZ(A)ANTHRACENE	
	50-32-8	BENZO(A)PYRENE	
	205-99-2	BENZO(B)FLUORANTHENE	
	192-97-2	BENZO(E)PYRENE	
	191-24-2	BENZO(G,H,I)PERYLENE	
	207-08-9	BENZO(K)FLUORANTHENE	
	92-52-4	BIPHENYL	
	218-01-9	CHRYSENE	
	53-70-3	DIBENZO(A,H)ANTHRACENE	
	206-44-0	FLUORANTHENE	
	86-73-7	FLUORENE	
	193-39-5	INDENO(1,2,3-C,D)PYRENE	
	91-20-3	NAPHTHALENE	
	198-55-0	PERYLENE	
	85-0108	PHENANTHRENE	
	129-00-0	PYRENE	
PCB	34883-43-7	2,4'-DICHLOROBIPHENYL	CL2(8)
	37680-65-2	2,2',5-TRICHLOROBIPHENYL	CL3(18)
	7012-37-5	2,4,4'-TRICHLOROBIPHENYL	CL3(28)
	41464-39-5	2,2',3,5'-TETRACHLOROBIPHENYL	CL4(44)
	35693-99-3	2,2',5,5'-TETRACHLOROBIPHENYL	CL4(52)
	32598-10-0	2,3',4,4'-TETRACHLOROBIPHENYL	CL4(66)
	32598-13-3	3,3',4,4'-TETRACHLOROBIPHENYL	CL4(77)
	37680-73-2	2,2',4,5,5'-PENTACHLOROBIPHENYL	CL5(101)
	32598-14-4	2,3,3',4,4'-PENTACHLOROBIPHENYL	CL5(105)
	31508-00-6	2,3',4,4',5-PENTACHLOROBIPHENYL	CL5(118)
	57465-28-8	3,3',4,4',5-PENTACHLOROBIPHENYL	CL5(126)
	38380-07-3	2,2',3,3',4,4'-HEXACHLOROBIPHENYL	CL6(128)
	35065-28-2	2,2',3,4,4',5-HEXACHLOROBIPHENYL	CL6(138)
	35065-27-1	2,2',4,4',5,5'-HEXACHLOROBIPHENYL	CL6(153)
	35065-30-6	2,2',3,3',4,4',5-HEPTACHLOROBIPHENYL	CL7(170)
	35065-29-3	2,2',3,4,4',5,5'-HEPTACHLOROBIPHENYL	CL7(180)
	52663-68-0	2,2',3,4',5,5',6-HEPTACHLOROBIPHENYL	CL7(187)
	52663-78-2	2,2',3,3',4,4',5,6-OCTACHLOROBIPHENYL	CL8(195)
	40186-72-9	2,2',3,3',4,4',5,5',6-NONACHLOROBIPHENYL	CL9(206)
	2051-24-3	DECACHLOROBIPHENYL	CL10(209)

- Data are segregated by species using the first ten characters of the MATRIX_CODE in the SAMPLE table and the FRACTION_CODE in the bottle table.

Table 3. Matrix and Fraction Codes Used in Threshold Calculations.

Species	MATRIX_CODE	FRACTION_CODE
Lobster	6181010201	MEAT
Flounder	8857041504	FILLET
Mussel	5507010101	SOFT_TISSUE

- Data are stored in the database as a dry weight concentration. The thresholds are based on lipid normalized values or wet weight percentages. A conversion is made using the measurement of percent dry weight and percent lipids. These values are stored in the ANALYTICAL_RESULTS table with PARAM_CODES of 'PCTDRYWT' and 'LIPID'.

Flounder Liver Disease:

- Centrotubular hydropic vacuolation severities (PARAM_CODE = 'CENTRO_HV') are used to measure flounder liver disease. These data are stored in the PATHOLOGY table with supporting data in the BOTTLE and SAMPLE tables.
- The samples are identified by the MATRIX_CODE for flounder shown in table 3 and a FRACTION_CODE of 'LIVER_SECTION'.

Data to be used in the analysis:

Tissue Body Burdens:

- Data from the outfall station are used for baseline and threshold testing. (STAT_ID = '4' for flounder and lobster, STAT_ID = '4M' for mussels).
- The baseline years are shown in table 1. One year, 1992, was excluded from the baseline calculation for flounder because the compositing scheme is incompatible with subsequent years.

Flounder Liver Disease:

- Data from the Deer Island Flats station are used for baseline calculation (STAT_ID = '1')
- Data from the Outfall Site are used for threshold testing calculation. (STAT_ID = '4').
- Data from 1991 to 2000 was used in calculating the baseline value.

Both Analyses:

- Data qualified as invalid/suspect (those having qualifiers including 's') are excluded. No other exclusions are made. The existence of data that are qualified as "possibly suspect/invalid, investigation pending" (those having qualifiers including 'q') will prevent a calculation from occurring.
- Data qualified as below detection limit ('a' qualifier) are treated as zero values.

Data Aggregation:

Tissue Body Burdens:

- The average of all analytical replicates for each parameter is calculated for each bottle (subsample).
- The average of all bottle averages for each parameter is calculated for each sample. A sample is a composite of flounder, lobster, or mussels comprising some of the individuals from a station, composited as described in the combined work/quality assurance project plan for Fish and shellfish monitoring (see Lefkowitz and Moore, 1998, ENQUAD report ms-049.)
- The sample average for each parameter is converted to a lipid normalized value or a wet weight value using the sample average percent dry weight or lipid percent dry weight value. The unit code in Table 1 indicates how each parameter is treated.

  Lipid Normalized Value = SAMPLE_AVERAGE * 100 / SAMPLE_AVERAGE_LIPID
  Wet Weight Value = SAMPLE_AVERAGE * SAMPLE_AVERAGE_PCTDRYWT / 100
- Annual averages for each parameter are calculated by averaging across samples for a given year for each parameter.
- The annual values for chlordane, DDT, PCB, and PAH are calculated by summing the annual averages of the parameters listed in table 2.

Flounder Liver Disease:

- If any liver section in a given flounder sample has a centrotubular hydropic vacuolation count greater than 0, the sample is assigned a value designating it as diseased; otherwise it is assigned a value designating it as nondiseased.
- The annual percent prevalence is computed as 100 times the number of fish with disease divided by the total number of fish.

Baseline Calculation (FFLM_BASE.SQL):

Tissue Body Burdens:

- The average of annual values for all years shown in table 1 are used to calculate the baseline average for chlordane, DDT, dieldrin, and PAHs.
- The threshold value for these groups is calculated as twice the baseline average.

Flounder Liver Disease:

- The average of the annual percent prevalence for the Deer Island Flats station (station 1) is the baseline average and the threshold value.

Threshold Testing (FFLM.SQL):

Tissue Body Burdens:

- The annual value for each post-discharge year (reported to three significant figures) is compared to the threshold value. If the annual value is greater, a threshold exceedance is recorded.

Flounder Liver Disease:

- The annual percent prevalence at station 4 for each post-discharge year is compared to the threshold value and, if it is greater, an exceedance is recorded.

Approvals:

Written by:

	_____	_____
	Suh Yuen Liang	Date
	_____	_____
	Joseph LoBuglio	Date

Data Group Manager:

	_____	_____
	Wendy Leo	Date

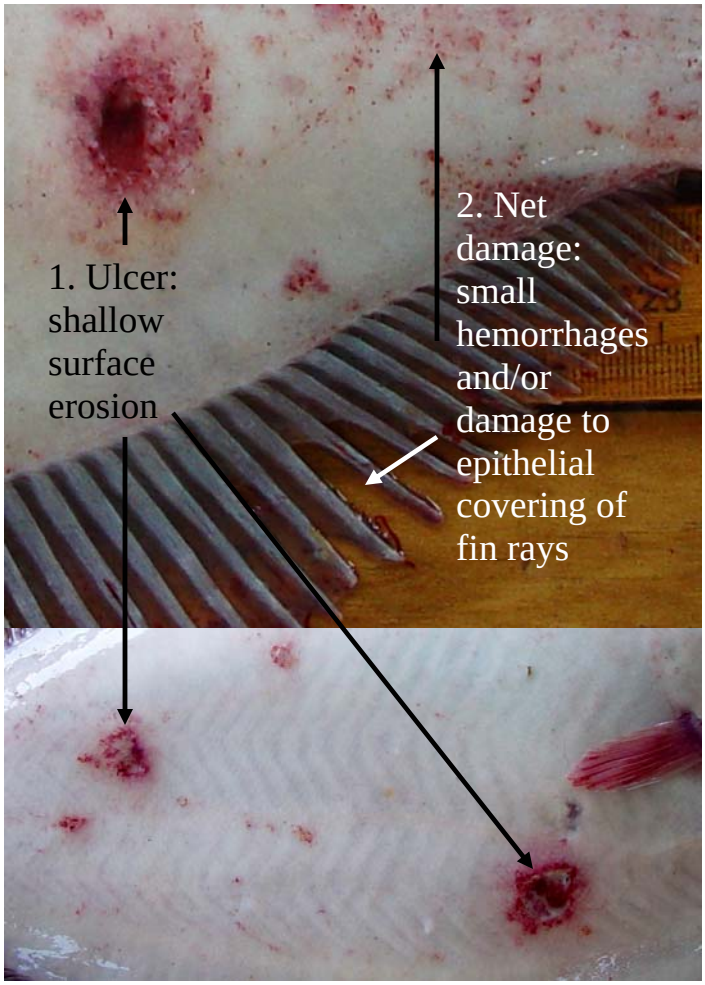
MWRA Manager Responsible for Fish and Shellfish Data:

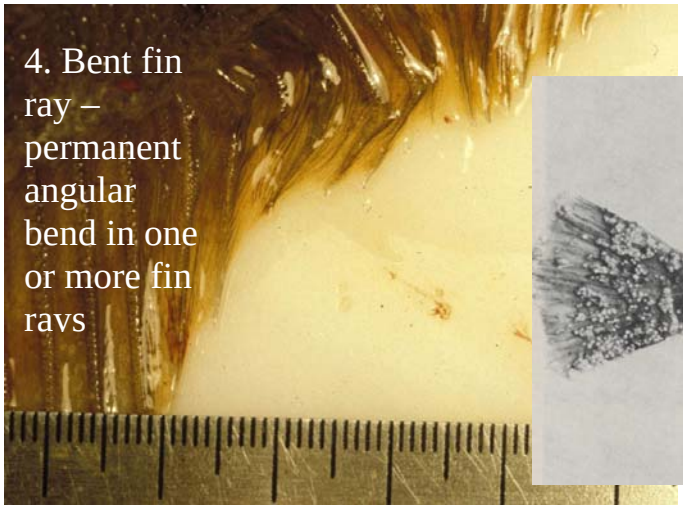
	_____	_____
	Maurice Hall	Date

Appendix B

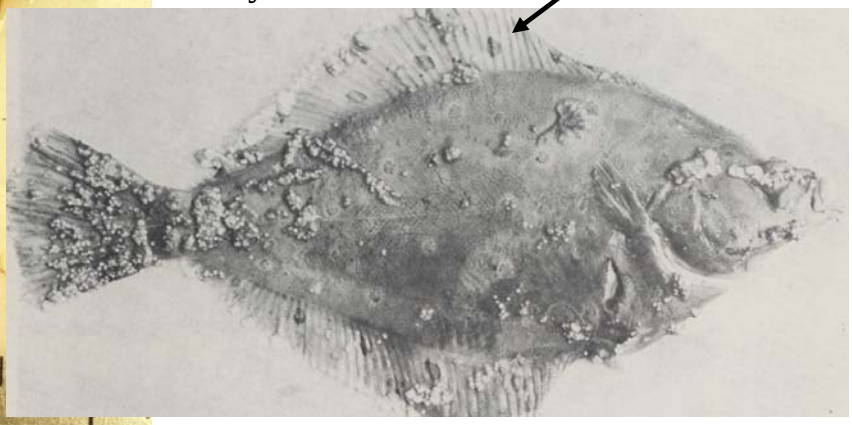
Guidance for Recording External Lesions in Flounder

SCORING EXTERNAL LESIONS ON WINTER FLOUNDER





5. Lymphocystis - ~2 mm spheres on fish surface or more typically attached to fin rays



Plaice - Sinderman et al 1978

Grade the severity of the lesions present. The severity grade should be an aggregate estimate of how severely each fish is affected overall with a particular lesion type. Lesion severity should be estimated on a range of 0: absent, 1: mild, 2: moderate, 3: severe and 4; extreme. Record date, time and latitude and longitude of sample. The ulcer, fin erosion and lymphocystis cases would be a severity 4.

Winter flounder external lesion record. Grade each fish for overall lesions severity for each lesion type

Date	Latitude	Longitude	Ulcer	Net damage	Fin erosion	Bent fin ray	Lymphocystis	Other

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