Quality Assurance Project Plan (QAPP)

for

Winter Flounder Monitoring: 2010

Massachusetts Water Resources Authority
Environmental Quality Department
Report 2010-07

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QUALITY ASSURANCE PROJECT PLAN (QAPP)

for

Winter Flounder Monitoring: 2010

Tasks 2.0, 3.2, 4.0, 8.1, 9.1, and 11.0, and 12.3

MWRA Harbor and Outfall Monitoring Project
Contract No. OP118B

Prepared for

Massachusetts Water Resources Authority
Environmental Quality Department
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May 2010
Report No. 2010-7

A. PROJECT MANAGEMENT

VERSION 0

A1. TITLE AND APPROVALS

QUALITY ASSURANCE PROJECT PLAN (QAPP) *for* WINTER FLOUNDER MONITORING: 2010

MWRA Harbor and Outfall Monitoring Project
Contract No. OP118B

Prepared by
AECOM Marine & Coastal Center
and
Woods Hole Oceanographic Institution
May 2010
Review and Approvals

James A. Blake, Ph.D. AECOM Program Manager	Date
Donald Galya, P.E. AECOM Principal-in-Charge	Date
Ms. Debra Simmons AECOM Project QA Officer	Date
Michael Moore, Ph.D. WHOI Principal Investigator	Date
Mr. Kenneth E. Keay MWRA Project Manager	Date
Mr. Maurice Hall MWRA Project Area Manager	Date
Ms. Wendy Leo MWRA EM & MS Manager	Date

A2. TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

APPENDIX

A3. DISTRIBUTION LIST

Copies of this QAPP, and any subsequent revisions, will be distributed after approvals have been obtained. Copies will be sent to the following personnel:

Name	Date Sent
Kenneth E. Keay, MWRA	
Maurice Hall, MWRA	
Wendy Leo, MWRA	
Michael Moore, WHOI	
James A. Blake, AECOM	
Debra Simmons, AECOM	
Pamela A. Neubert, AECOM	
Stacy A. Doner, AECOM	
Paula Winchell, AECOM	
Vivian English, Experimental Pathology Laboratories	
Jay Burnett, NMFS Woods Hole	
Greg Lescarbeau, Battelle	

A4. PROJECT AND TASK ORGANIZATION

The Winter Flounder (*Pseudopleuronectes americanus*) Monitoring tasks will be accomplished through the coordinated efforts of experienced personnel from AECOM, Woods Hole Oceanographic Institution (WHOI), Experimental Pathology Laboratories (EPL), and Battelle.

MWRA

The following MWRA managers will be informed of all matters pertaining to work described in this QAPP.

- Dr. Andrea Rex, Director of the MWRA Environmental Quality Department (ENQUAD).
- Mr. Ken Keay, MWRA Harbor and Outfall Monitoring Program (HOM) Project Manager. Mr. Keay has primary administrative and budgetary oversight of the program.
- Mr. Maurice Hall, MWRA Project Area Manager for Fish & Shellfish, including Winter Flounder Monitoring.
- Ms. Wendy Leo, MWRA Environmental Monitoring and Management System (EM&MS) Database Manager.

AECOM

- Dr. James A. Blake, AECOM Program Manager, is responsible for the overall performance of this project and for ensuring that products and services that meet MWRA's expectations are delivered in a timely and cost-effective manner. He is responsible for ensuring that data collection and interpretation are scientifically defensible and for responding to technical challenges as they arise.
- Mr. Donald Galya, AECOM Principal-in-Charge, will be responsible for providing overall direction and coordination of the project, ensuring that project goals are achieved, and providing adequate resources to the project manager and management team.
- Ms. Debra Simmons, AECOM Project Quality Assurance (QA) Officer, is responsible for reviewing the QAPP, survey reports, and data reports. She will also review QA Statements submitted by subcontractors for quality, completeness, and adherence to the QAPP.
- Mr. Philip Platcow, AECOM's Health & Safety Officer, will review and approve the health and safety plans and procedures for the project.
- Ms. Paula Winchell will participate in QAPP preparation, in field sampling, and will be AECOM's On-site Health & Safety Officer. She will oversee local and on-site health and safety procedures for the project.
- Dr. Pamela L.A. Neubert is AECOM's Deputy Program Manager for the HOM7 contract and Task Leader for the field survey.
- Ms. Stacy A. Doner is the AECOM Task Manager for the laboratory and data portions of the flounder monitoring task.
- Dr. Nancy J. Maciolek will prepare the QAPP and assist Dr. Moore with data analysis and report preparation.

WHOI

- Dr. Michael Moore is the Principal Investigator for the Flounder Monitoring Task. Dr. Moore will examine the histological slides, analyze and reduce the histological data, and add the data to the ongoing temporal and spatial data summaries. Dr. Moore will prepare the annual reports on

the results of the flounder tissue analysis. Dr. Moore is also the Chief Scientist for the flounder survey and in this role will ensure that field activities are implemented in accordance with the QAPP and survey plan.

BATTELLE

- Mr. Gregory Lescarbeau, Battelle, will oversee the data loading for the flounder survey and flounder histology tasks.

Additional Participants

- Dr. Jay Burnett, NMFS Woods Hole, will analyze the fish scales to determine age of the specimens.
- Mr. Mark Carrol, Captain of the F/V *Harvest Moon*, will provide navigational support for the field surveys.
- Ms. Vivian English, EPL, will oversee the preparation of histological slides under subcontract to WHOI.

Addresses, telephone and fax numbers, and Internet addresses, as well as specific project roles and responsibilities for project participants, are summarized in Table 1.

The following tasks covered by Contract OP118B are relevant to Winter Flounder Monitoring:

- Task 2.0 Project Management
- Task 3.2 Development of QAPP
- Task 4.0 Data Quality Control and Data Set Submission
- Task 8.1 Flounder Field Survey
- Task 9.1 Flounder Histological Analysis
- Task 11.0 Annual Technical Meeting
- Task 12.3 Synthesis Report

The Management task (2.0) is not further addressed. The deliverable for 3.2 (QAPP) is described in section A.9.4.1. Tasks 4, 8.1, 9.1, and 12.3 are discussed in detail in section A6 of this QAPP. Task 11.0 includes participation in an Annual Technical Workshop and is not further described.

Table 1. Personnel Responsibilities and Contact Information for Flounder Monitoring.

Name/ Affiliation	Address	Project Area Assignment	Contact Information
MWRA			
Dr. Andrea Rex	Environmental Quality Department MWRA Charlestown Navy Yard 100 First Ave. Boston, MA 02129	Director of Environmental Quality Department	Ph: 617-788-4940 Fax: 617-788-4888 andrea.rex[at]mwra.state.ma.us
Mr. Kenneth Keay		Project Manager	Ph: 617-788-4747 Fax: 617-788-4888 kenneth.keay[at]mwra.state.ma.us
Mr. Maurice Hall		Fish and Shellfish Project Area Manager	Ph: 617-788-4944 Fax: 617-788-4888 maurice.hall[at]mwra.state.ma.us
Ms. Wendy Leo		EM&MS Manager	Ph: 617-788-4948 Fax: 617-788-4888 wendy.leo[at]mwra.state.ma.us
AECOM			
Dr. James A. Blake	AECOM Marine and Coastal Center 89 Water St. Woods Hole, MA 02543	Program Manager **(All Tasks)**	Ph: (508) 457-7900 Fax: (508) 457-7595 James.Blake[at]aecom.com
Dr. Pamela L.A. Neubert		Deputy Program Manager; Task Manager – Field Operations **(Task 8.1)**	Ph: (508) 457-7900 Fax: (508) 457-7595 Pamela.Neubert[at]aecom.com
Dr. Nancy J. Maciolek		Editor, QAPP (**Task 3.2**) and Synthesis Report **(Task 12.3)**	Ph: (508) 457-7900 (AECOM) Ph: (781)585-5822 (direct) Fax: (508) 457-7595 njmaciolek[at]gmail.com
Ms. Stacy A. Doner		Task Manager – Flounder Data; QA Documentation and Transmission; **(Task 4)**	Ph: (508) 457-7900 Fax: (508) 457-7595 Stacy.Doner[at]aecom.com
Ms. Paula Winchell		QAPP preparation (**Task 3.2**), Field (**Task 8.1**)	Ph: (508) 457-7900 Fax: (508) 457-7595 Paula.Winchell[at]aecom.com
Ms. Debra Simmons	AECOM 2 Technology Park Drive Westford, MA 01886	Project QA Officer; **(Tasks 3.2 and 4)**	Ph: (978) 589-3358 Fax: (978) 589-3035 Debbie.Simmons[at]aecom.com
Mr. Philip A. Platcow	AECOM 66 Long Wharf Boston, MA 02110	Project Health and Safety Officer	Ph: (617) 371-4461 Cell: (617) 899-5403 Philip.Platcow[at]aecom.com

Table 1, continued.

Name/ Affiliation	Address	Project Area Assignment	Contact Information
WHOI			
Dr. Michael Moore	Woods Hole Oceanographic Institution Mail Stop 50 Woods Hole, MA 02543	Chief Scientist, Flounder Survey Principal Investigator, Histology of flounder tissue, laboratory and data analysis, interpretation and presentation of results. **(Tasks 3.2, 4, 8.1, 9.1, 11, and 12.3)**	Ph: (508) 289-3228 Ph: (508) 989-3575 (cell) Fax: 508 457 2089 mmoore[at]whoi.edu
Battelle			
Mr. Gregory Lescarbeau	Battelle Duxbury Operations 397 Washington St. Duxbury, MA 02332	Manager, Database Support **(Task 4)**	Ph: (781) 952-5293 Fax: (781) 934-2124 lescarbeaug[at]battelle.org
Additional Subcontractors			
Mr. Mark Carrol	Not available at this time.	Captain, F/V *Harvest Moon* **(Task 8.1)**	(978) 985-7645
Dr. Jay Burnett	NMFS, Woods Hole	Scale analysis (**Task 9.1)**	Ph: (508)-495-2000 x 2286 jay.burnett[at]noaa.gov
Ms. Vivian English	Experimental Pathology Laboratories, P.O. Box 474, Herndon, VA 20172	Histological preparation (**Task 9.1**)	Ph: (703) 471-7060 x222

A5. PROBLEM DEFINITION AND BACKGROUND

A5.1 Background

Boston Harbor has a long history of anthropogenic impacts, including the direct discharge of sewage waste products into the harbor, resulting in many discernable impacts on the biology of the harbor. One such impact was the high prevalence of fin rot and lesions in bottom-dwelling flounder, *Pseudopleuronectes americanus*: in the mid-1980s, Boston Harbor flounder were identified as having among the highest incidence of liver tumors in the northeastern United States (Murchelano and Wolke 1985).

In 1972, the Federal Clean Water Act (CWA) mandated secondary treatment for all sewage discharges to coastal waters, but an amendment allowed communities to apply for waivers from this requirement. The metropolitan Boston area's waiver application was denied by the US Environmental Protection Agency (EPA) and in 1985, in response to both the EPA mandate to institute secondary treatment and a Federal Court order to improve the condition of Boston Harbor, the Massachusetts Water Resources Authority (MWRA) was created.

The MWRA instituted a multifaceted approach to upgrading the sewage treatment system, including an upgrade in the treatment facility itself and construction of a new outfall pipe to carry the treated effluent to a diffuser system in Massachusetts Bay. In September 2000, the effluent from Deer Island was diverted to a new outfall approximately 15 km offshore, in 32 m water depth in Massachusetts Bay. The offshore outfall is regulated under a permit issued to MWRA by EPA and the Massachusetts Department of Environmental Protection (DEP), under the National Pollutant Discharge Elimination System (NPDES).

One requirement of the permit is that the MWRA conduct a monitoring program to track responses of several environmental factors; warning-level thresholds for these factors are listed in a Contingency Plan that also details the process of how the MWRA would respond to any exceedences of the threshold values (MWRA 2001). The Contingency Plan is an attachment to the Memorandum of Agreement among the National Marine Fisheries Service, USEPA, and MWRA. Warning-level thresholds listed in the plan are based on effluent limits, observations from baseline monitoring, national water quality criteria, state standards, and, in some cases, best professional judgment. Thresholds have been established for the incidence of flounder liver disease and for organic and inorganic levels of tissue contaminants (MWRA 2001).

A5.2 Flounder Monitoring

The MWRA Harbor and Outfall Monitoring (HOM) program includes a winter flounder monitoring component, which supports the collection of data on the external condition of winter flounder and on the body burden of selected anthropogenic contaminants in the liver and edible tissue. Fish are collected from a wide geographic area that is influenced by many natural and anthropogenic factors including past and current discharge of effluents from MWRA wastewater outfalls. This monitoring provides valuable information on the temporal responses of flounder at the outfall in Massachusetts Bay and in the now cleaner waters of Boston Harbor.

After the discontinuation of sludge deposition in the harbor in 1991, the flounder caught near Deer Island generally began to appear healthier and did not exhibit the gross abnormalities, such as fin erosion, that had been observed during the mid-1980s. However, in 2006 fin erosion occurred in more than half the fish taken from Deer Island Flats, the highest incidence seen since the early days of the monitoring program but within the historic range (Werme et al. 2007).

Centrotubular hydropic vacuolation (CHV) has been the most common type of liver lesion seen. By 2002, the rate of CHV was about two-thirds of the levels found in the 1980s, although this might be partially explained by the young age of the fish, since younger fish tend to have lower CHV levels. In 2006, incidence of CHV in fish from the outfall site was comparable to levels observed in 2001 and 2005 and lower than the incidence in the years before the outfall began to discharge (Werme et al. 2007). In recent years, there has been an increase in percent CHV (Figure 1), but levels are not considered to be significant at this time (Moore et al. 2010). Liver tumors, which indicate more serious health effects, have not been observed since 1996 (Rex et al. 2002).

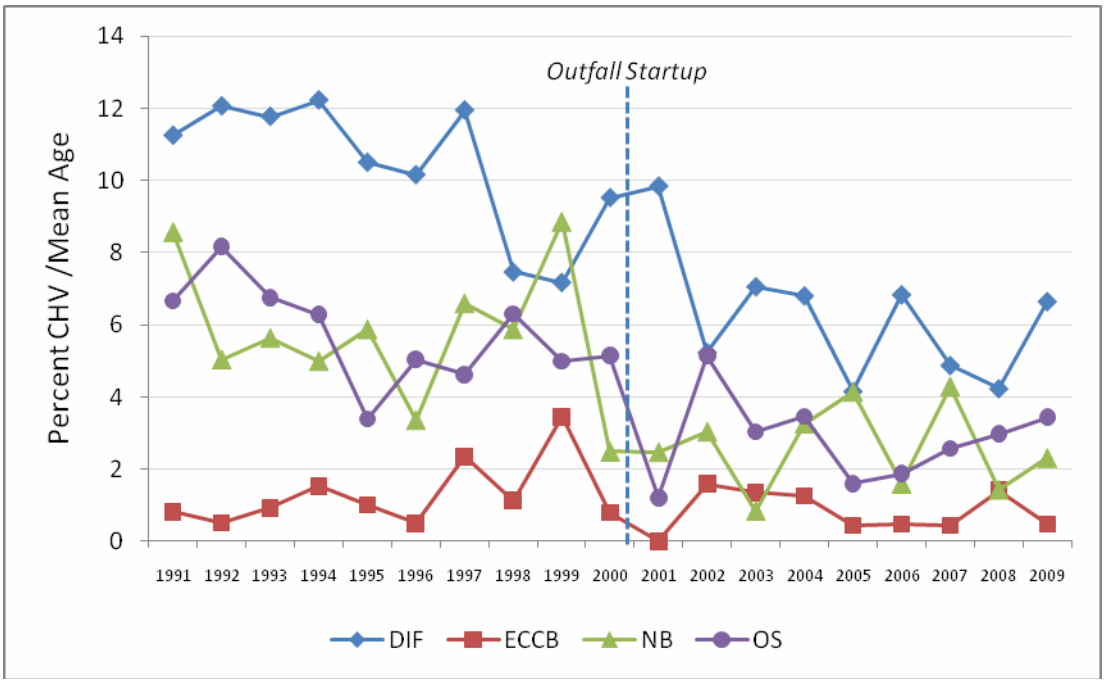

Figure 1. Prevalence of centrotubular hydropic vacuolation (CHV), corrected for age. DIF =Deer Island Flats, NB = Nantasket Beach, BS = Broad Sound, OS = Outfall Site, and ECCB = Eastern Cape Cod Bay. From *Werme et al. 2007*.

In the April 2003 flounder survey, a significant prevalence of blind-side ulcerative dermatitis was recorded, with the highest prevalence in western Massachusetts Bay (Moore 2003). Additional pathology and microbiology studies were unable to determine a cause of the ulcers (Moore et al. 2004). Additional surveys conducted throughout 2004 and 2005 established that ulcer prevalence peaked in late winter to early spring, with evidence of healed ulcers and lower ulcer prevalence into early summer (Moore 2006). This apparent recovery sequence suggests that these lesions may be a non-lethal seasonal condition. Ulcer prevalence in the 2009 survey suggested a continuing decrease over recent years, declining at the outfall site from 36% in 2004 to 0% in 2009.

Concentrations of contaminants in flounder fillets have remained low, with the highest levels of most contaminants observed in fish from Deer Island Flats. Concentrations of contaminants in flounder livers have shown a similar pattern. Statistical analyses of data from the years immediately prior to the outfall

coming on line (1998–2000) in comparison to those from post-discharge years (2001–2003 and 2006) found no increase in contaminant levels in flounder fillets. Chlordane levels (alpha-chlordane + trans-nonachlor) have significantly decreased in the years following outfall start-up. There were increases in PCB concentrations, measured as congeners 138+153, in flounder livers from fish from the outfall site and Deer Island flats, but the levels were within historic ranges (Werme et al. 2007).

A5.3 Objectives and Scope

The overall objective of MWRA's winter flounder monitoring program is to define the condition of flounder health in terms of the presence of disease (external and internal). In some years, such as 2009 but not 2010, the organic and inorganic contaminant levels in the edible tissue are measured.

The objectives of this project are addressed in three major tasks to be conducted under the present contract. Task 8.1 comprises sampling activities in Boston Harbor, Massachusetts Bay, and Cape Cod Bay. Task 9.1 includes the histological and age analysis of the flounder collected from each site and the data report from that analysis. An interpretative report that includes a synthesis of the histological data is included in Task 12.3. Results will be evaluated against established monitoring thresholds and will include an update and discussion of long-term trends in flounder health as determined by the monitoring studies in Boston Harbor and Massachusetts Bay. An oral presentation of results will be made at the Annual Technical Workshop (Task 11).

A6. PROJECT/TASK DESCRIPTION

A6.1 Task 8.1: Winter Flounder Survey

Trawls will be made in late April and/or early May 2010 at four locations: Deer Island Flats, off Nantasket Beach, near the offshore outfall site, and Eastern Cape Cod Bay (Figure 2, Table 2). Fifty sexually mature (i.e., >300 mm total length) winter flounder (*Pseudopleuronectes americanus*) will be collected at each station. If lack of fish at any one station precludes a sample of 50 sexually mature individuals after a reasonable effort (e.g., repeated efforts with multiple commercial otter trawls), then the MWRA Project Manager will be notified of these circumstances and may accept the survey as 100% complete. Alternatively, a second collecting trip may be scheduled in order to obtain the full number of fish required at each station, as was done in 2009 (Moore et al. 2010).

The Catch Per Unit Effort (CPUE) (the number of fish caught per minute of bottom time) will be calculated at each sampling station. Upon collection, flounder will be examined and the external condition of each will be noted, especially any abnormalities such as gross deformities or fin rot. Such abnormalities are scored from 0 to 4, where: 0 = absent; 1 = minor; 2 = moderate; 3 = severe; and 4 = extreme. Liver samples will be preserved for histopathological examination of the tissue.

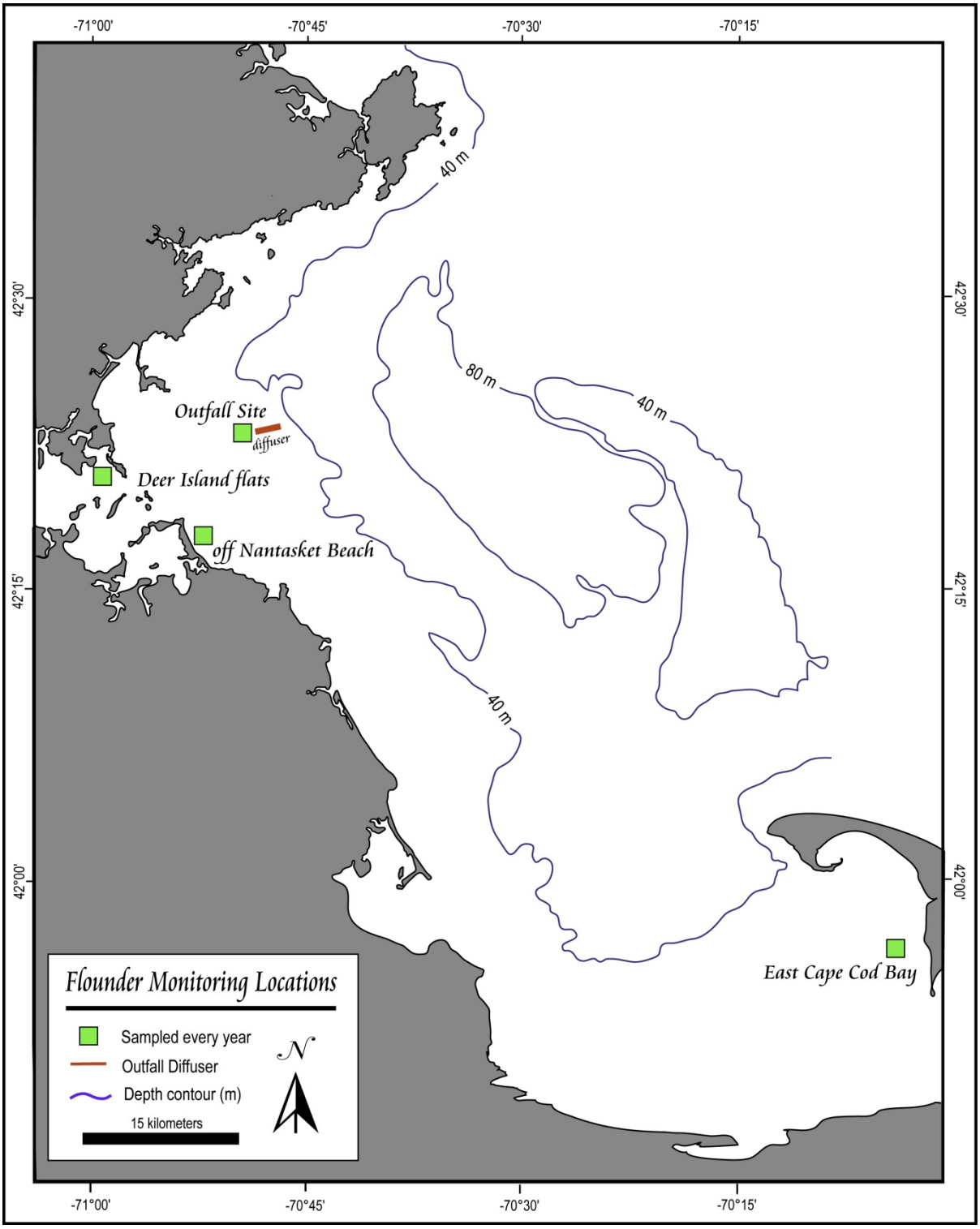

Figure 2. Flounder monitoring locations for 2010.

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′
OS	Outfall Site	42°23.1′	70°49.3′
ECCB	East Cape Cod Bay	41°56.2′	70°06.6′

A6.2 Task 9.1: Flounder Histology Analysis

An assessment of the health of flounder collected in Boston Harbor and Massachusetts Bay will be made through histological examination of liver tissue. Samples will be taken from the livers of the 50 flounder collected at each of the four survey sites sampled. Fixed specimens will be transferred to Experimental Pathology Laboratories in Herndon, VA, where they will be embedded in paraffin, sectioned at 5 µm and stained with hematoxylin and eosin using standard methods (Luna 1992). Flounder age will be determined by scale analysis performed by Mr. Jay Burnett (Woods Hole NMFS Age and Growth Unit).

Dr. Michael Moore will analyze the prepared slides using bright field illumination at 25×, 100×, and 200×, using a Zeiss Axioskop research microscope. After an initial survey of the material, the presence of the following lesions, which have been described in detail elsewhere (Lefkovitz et al. 2004; Pembroke et al. 2006) will be recorded:

- 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 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.

In addition, beginning in 2008, the presence of liver flukes has been recorded. They are scored as follows: 0 absent, 1 rare, 2 common, and 3 abundant.

Histological data for flounder from the Outfall Site will be submitted to MWRA within two months of the field survey, and data from all four stations will be submitted within 120 days after survey completion. MWRA will compare current results with the threshold values established in the Contingency Plan (Table 3). The majority of flounder thresholds pertain to levels of tissue contaminants, which are not being measured under this contract. Therefore, of the thresholds listed in Table 3, only the level of incidence of liver disease is relevant to this task.

Table 3. MWRA Threshold Values for Flounder Disease and Contaminants.

Threshold ID	Parameter	Unit of Measure*	Threshold Value		Baseline years	FDA action levels
			Caution	Warning		
FFLIVDIS	liver disease incidence	%	44.94	-	1991–2000	N/A
FFFCHL	lipid-normalized chlordane	ng/g lipid	484	-	1993–2000**	300 ng/g
FFFDDT	lipid-normalized DDT	ng/g lipid	1552	-	1993–2000**	5000 ng/g
FFFDIEL	lipid-normalized dieldrin	ng/g lipid	127	-	1993–2000**	300 ng/g
FFFHG	mercury	µg/g wet	0.5	0.8	N/A	1 µg/g
FFFPCB	PCB	ng/g wet	1000	1600	N/A	2000 ng/g

* = Lipid normalized thresholds are based on dry weights.
** = 1992 flounder data excluded because compositing scheme not compatible with other years.
N/A = Mercury and PCB thresholds not calculated using baseline data; there is no FDA action level for incidence of liver disease.

A6.3 Task 12.3: Flounder Report

Results of the histopathological analysis will be summarized and interpreted in an annual report, which will include the results of all activities carried out each year. Analysis of the data will include a comparison of the current year's results against baseline and will focus on evaluating the overall health of local flounder populations to determine if the discharge from the outfall is affecting this species. The report will include a general discussion of long-term trends in flounder health.

For each liver lesion type, the percent prevalence will be calculated based on the three liver sections from each fish at each station. 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. Analysis of variance will be used to compare lesions from site to site and annually.

A6.4 Schedule of Activities and Deliverables

Flounder monitoring activities under this contract will span the period from the date of project initiation (March 1, 2010) until completion of the final report in 2010. Activities include field sampling and laboratory analyses, with deliverables consisting of a QAPP, a survey plan, a survey summary, a survey report, sample analysis data submission, data report review, and annual report. Schedules for these activities and deliverables for 2010 are outlined in Table 4.

Table 4. Schedule of Flounder Monitoring Deliverables.

Task	Deliverable	Due Date
Quality Assurance Project Plan (Task 3.2)	QAPP	Draft: March 2010 Final: April 2010
Flounder Survey (Task 8.1)	Survey Plan Survey Cruise Survey Summary E-mail Survey Report	March 2010 April 2010 2 days after survey May 2010
Flounder Survey Data (Task 4)	Survey Data	May 2010
Flounder Histology Analysis (Tasks 4 and 9.1)	Histology Data	August 15 (preliminary report for liver disease incidence at Outfall Site is due 60 days after survey)
Flounder Histology Data Report Review (Task 9.1)	Letter Report of Review Comments	September 2010
Annual Flounder Report (Task 12.3)	Draft Report Final Report	September 2010 October 2010

A7. QUALITY OBJECTIVES AND CRITERIA

To ensure that data generated in this program are of a quality suitable for their intended use and are technically defensible, certain requirements for accuracy, precision, representativeness, comparability, and completeness must be met. These elements can be defined as:

Accuracy: The extent of agreement between a measured value and the true value of interest.

Precision: The extent of mutual agreement among independent, similar, or related measurements.

Representativeness: The extent to which measurements represent true systems.

Comparability: The extent to which data from one study can be compared directly to data from similar studies.

Completeness: The measure of the amount of data acquired versus the amount of data required to fulfill the statistical criteria for the intended use of the data.

The representativeness and comparability of all the data generated in this project depend to some extent on the selection of the sampling sites. All flounder monitoring stations to be visited during this program will be the same as those visited in previous years (Lefkovitz et al. 2004 (HOM4, though not including station BS – Broad Sound), Pembroke et al. 2006 (HOM5), Moore et al. 2008 (HOM 6)).

Quality objectives are given below. Details of how these criteria will be met for each component of the flounder monitoring tasks are presented in section B5 of this QAPP.

A7.1 Field Activities

A7.1.1 *Navigation*

The quality objective for navigation is that the system used be accurate and precise in order to enable the sampling vessel to reliably re-occupy those stations that are to be sampled during each survey. Although the Global Positioning System (GPS) navigation equipment should be accurate and suitable for consistently fixing the vessel's position to within 10 meters of a target station, the data quality objective for flounder sampling is that trawling occur in the general vicinity of the target, depending on where fish can be found. The target radius for each station is as follows: DIF, 3000 m; ECCB, 10,000 m; NB, 2000 m; and OS, 3000 m.

A7.1.2 *Flounder Collection*

The quality objectives for the collection of flounder are that (1) fish are properly identified to species and only specimens of winter flounder (*Pseudopleuronectes americanus*) are retained and (2) all required flounder (50 mature specimens) are collected at each station.

The quality objectives for the handling of flounder samples are that (1) all fish are externally examined and all abnormalities carefully noted, (2) samples are fixed in 10% formalin as quickly as possible to prevent deterioration of tissues, and (3) samples are labeled accurately. Procedures for sample handling are detailed in section B3 of this QAPP.

A7.2 Laboratory Activities

A7.2.1 *Histological Analysis*

The data quality objectives for the histological analysis of flounder livers are that (1) all samples are processed, (2) all laboratory methods as detailed in section B4 of this QAPP are followed, and (3) all data are obtained and are traceable and documented accurately.

A8. SPECIAL TRAINING AND CERTIFICATIONS

A8.1 Special Training

A8.1.1 *Field Activities*

Field personnel will be experienced in the sampling techniques documented in the QAPP. Prior to starting work, personnel will be given instructions specific to the project, covering the following areas:

- Organization and lines of communication and authority
- Overview of the QAPP
- QA/QC requirements
- Documentation requirements
- Health and safety requirements

Instructions will be provided and documented by the AECOM Program Manager, AECOM Health and Safety Officer, and AECOM Project QA Officer.

Personnel responsible for shipping samples will also be trained in the appropriate regulations, i.e., Department of Transportation (DOT), International Civil Aviation Organization (ICAO), and International Air Transport Association (IATA).

A8.1.2 *Laboratory Activities*

Personnel preparing histological sections of flounder tissues, including cutting, staining, and mounting, will be experienced in histological microtechnique. The senior scientist interpreting these preparations will be trained in histology and identification of normal and abnormal fish tissues including specific types of lesions. Age analysis based on flounder scales will be performed by personnel experienced in this technique.

After receipt of the flounder liver tissues, EPL will prepare them for histological examination using standard methods (Luna 1992). Samples will be transferred from formalin to water upon receipt. Subsections of the original tissues will be cut and then passed through a series of increasing concentrations of ethyl alcohol (ETOH) up to and including 100% ETOH. The tissues will then be rinsed at least twice in this concentration to ensure that all water is removed. Tissues will be cleared in toluene or xylene prior to imbedding in paraffin. After the paraffin has hardened, blocks will be cut and prepared for sectioning on a microtome. Sections of each tissue will be cut to a thickness of 5 μm and mounted on slides using egg albumin as a medium. Slides will then be processed through toluene or xylene and a series of decreasing ETOH concentrations until a 100% aqueous solution is reached, at which time the tissues will be stained in hematoxylin. Counterstaining with Eosin or Fast Green will complete the process and the slides will then be returned to toluene or xylene where a mounting medium and coverslip will be applied. These fully processed slides will then be forwarded to Dr. Moore for examination.

Fish will be aged by counting the rings or annuli visible in the fish scales according to the methods of Fields (1988).

A8.2 Certifications

No special certifications are required for the work covered under this QAPP.

A9. DOCUMENTS AND RECORDS

A9.1 Documentation

Data collected in the field will be recorded by hand onto established data forms. All data collection notes will be made in permanent ink, initialed, and dated, and no erasures or obliterations will be made. If an incorrect entry is made, the information will be crossed out with a single strike mark and the correct entry will be made, initialed, and dated by the person making the correction. Completed data forms or other types of hand-entered data will be signed and dated by the individual entering the data. After the survey, the field data will be transcribed into an Excel spreadsheet designed by Battelle to be integrated with an Access loading application. Battelle will produce csv files for AECOM review that will be suitable for loading by MWRA into their database.

All data developed in the laboratory will be captured electronically. Corrections to electronic data in the laboratory will be documented on a hard-copy of the data. Direct-entry and electronic data entries will indicate the person entering the data. It will be the responsibility of Dr. Moore to ensure that all data entries and hand calculations are verified according to the procedures described in sections D-1 and D-2 of this QAPP. Dr. Moore will provide a QA Report with his data deliverables.

A9.2 Field Records

Field sampling log sheets will provide the primary means of recording the data collection activities performed during the sampling activities. Dr. Moore, as Chief Scientist, is responsible for ensuring that all events occurring during the survey are adequately documented in the Survey Log. All data collected during the survey will be recorded on Station Log forms (See Figures 3 and 5). The start/end tow times and vessel position will be recorded by hand from the on-board GPS navigation system. At the end of the survey, Dr. Moore will provide copies of the completed survey records to AECOM for the HOM7 project files and to Mr. Hall (MWRA) for review. A separate log book is maintained by the Chief Scientist, in which he will record marine mammal observations, any problems with the fishing gear, navigation, or other sampling equipment, and in general any deviations from the QAPP. Dr. Moore transmits any relevant field information to AECOM for inclusion in the Survey Report.

Chain-of-custody forms will be used to track samples as described in section B3.2. The original forms will be returned to AECOM for the HOM7 project files once sample processing is complete.

A9.3 Laboratory Records and Deliverables

There are three sources of data for this project: (1) survey data, including station data and external information on each of the 200 fish collected, are recorded by hand on log sheets in the field, (2) age analysis data provided by Mr. Jay Burnett at NOAA are recorded by hand and provided to Dr. Moore, and (3) histology data read by Dr. Moore from the slides and recorded electronically into an Access loading application provided by Battelle.

The survey data (item 1 above) consist mostly of station identifications, dates and times, and navigation data and will be entered by Dr. Moore into an Excel file provided by Battelle and prepared for the Survey Report. The field data on fish from (1) will be entered into the Access loading application together with age analysis data (item 2 above) and the histology data (item 3 above).

After all data are entered, Dr. Moore will submit the loading application to AECOM for review. Ms. Stacy Doner will review the file and ensure that all required fields have been populated and will compare the electronic data with log sheets and other raw data supplied by Dr. Moore. This data file will then be forwarded to Mr. Greg Lescarbeau at Battelle for processing. A csv file will be generated by Battelle for AECOM that will be acceptable for loading by MWRA according to their rules. The csv file will be reviewed by Ms. Doner to ensure that all required fields have been properly converted from the Access application. Dr. Blake will then submit the file electronically to the MWRA Project Manager. Hard-copy of Dr. Moore's QA Report and other required information will be forwarded separately, together with any technical observations that Dr. Moore provides to accompany the report.

Data deliverables will be provided to MWRA by AECOM on the schedule described in this QAPP (Table 4, Section A6.4). Data management is discussed in section B10 of this QAPP.

A9.4 Reports and Data Submissions

Documents, data submissions, and reviews (with task numbers) that will be generated under flounder monitoring tasks are listed below. The due dates for these reports and data submissions are tabulated in Table 4 (section A6.4).

- QAPP (Task 3.2)
- Survey plan (Task 8.1)
- Survey summary (Task 8.1)
- Survey data submission (Task 4.13)
- Survey report (Task 8.1)
- Sample analysis data submissions (Tasks 4.14 and 9.1)
- Review of MWRA generated data report (Task 9.1)
- Annual report (Task 12.3)

A9.4.1 *QAPP*

The QAPP will be the first document produced for the Flounder Monitoring task and will be organized in the format documented in U.S. EPA QA/R-5 (2001, reissued 2006) and further elucidated in U.S. EPA QA/G-5 (2002). Copies, either electronic or hardcopy, of this QAPP, and any subsequent revisions, will be distributed by the AECOM QA Officer or the officer's designee to the personnel shown on the distribution list in section A.3 of this document. The revision number and date are given in the header.

A9.4.2 *Survey Plan*

A survey plan will be prepared for the survey and will be submitted as a final unbound, double-sided copy on 3-hole punched paper at least one week prior to the start of the survey. The survey plan will include the following information:

- General information
- Schedule of operations
- Background information
- Justifications and rationale
- Objectives
- Specific location and coordinates of each station
- Survey/sampling methods
- Sample handling and custody procedures
- Sequence of tasks and events
- Navigation and positioning control
- Vessel, equipment, and supplies
- QA/QC procedures
- Documentation procedures
- Scientific party
- Reporting requirements
- Safety procedures
- Documentation of any proposed modification from this QAPP

A9.4.3 *Survey Summary*

An e-mail summary will be delivered to the MWRA Project Area Manager, Mr. Maurice Hall, within two business days of survey completion. The summary will confirm completion of the survey and mention any noteworthy problems or events encountered. This summary will highlight any apparent or potential triggering of monitoring thresholds.

A9.4.4 *Survey Report*

A survey report will be prepared and submitted within one month after the survey. Two unbound, double-sided copies of the draft survey report will be submitted to MWRA, and comments will be due two weeks after receipt of the draft report. One unbound copy (double-sided on three-hole punched paper) of the final survey report, addressing MWRA's comments, will be due two weeks after receipt of the comments. If MWRA does not submit comments within the two-week period, the draft survey report will be considered final.

The report is expected to include about 4–5 pages of text, and will contain the following information:

- Introduction with overview of the survey, including the vessel, schedule, and a table of survey personnel (including roles and responsibilities)
- Survey chronology using local time
- Location of trawl lines with starting and ending coordinates and time on station
- Trawl recovery success
- Summaries of individual flounder measurements and gross conditions noted in the field
- Sufficiency of collected flounder to support biological (and chemical) analyses
- Number of specimens dissected and the disposition of the tissue samples
- Survey results presented as a narrative and including:
    - Any incidental observations of marine mammals
    - Any unusual observations of environmental conditions (especially those that might impact subsequent testing of Contingency Plan Thresholds)
    - Map illustrating the actual station locations and track lines
- Problems experienced, actions taken, and recommendations, including deviations from this QAPP that were not known at the time the survey plan was prepared
- References

All survey reports will include a station data table containing information specific to each individual survey (including, but not limited to, survey_ID, survey date, sampling times, sample types, sample locations, etc.). This data table will be generated by MWRA from the EM&MS database once the relevant survey data submission meets the QA criteria described in section B5 of this QAPP.

A9.4.5 *Data Reports*

Data from flounder collected at the Offshore Outfall Site are due to MWRA 60 days after survey completion. MWRA will calculate the Contingency Plan threshold for lesion prevalence at the outfall site based on these data. This preliminary report will be replaced by the complete data report in August or within 120 days after survey completion. MWRA will perform range checks on the data (see section B10.5) and will provide a draft report to AECOM and WHOI for review. AECOM will submit a letter to MWRA with comments on the draft.

A9.4.6 *Annual Flounder Report*

The annual report will summarize data generated for age, length, weight, sex, CPUE, external condition parameters, and the results of the histopathological study of the flounder liver tissue. Analysis of the data will focus on evaluating the overall health of local flounder populations to determine if the discharge from the outfall is affecting them. All project data used in the annual reports will be derived from the MWRA EM&MS database. The long-term flounder histology database dates from annual sampling starting in 1987 for the Deer Island Flats station in Boston Harbor and 1991 for the other three stations.

The report will include at a minimum:

- The CPUE, defined as the number of fish obtained per minute of bottom trawling time
- Life history observations such as the age, sex, and length (size) of fish caught
- Spatial and temporal trends in liver lesions, especially hydropic vacuolation and hepatic tumors which may indicate toxic effects on fish health
- Details of external pathology (condition), including surface ulcers such as those detected in 2003
- General discussion and update of long-term trends in flounder studies in Boston Harbor and Massachusetts Bay

A9.5 Project Files

The project files will be the central repository for all documents relevant to sampling and analysis activities as described in this QAPP. AECOM is the custodian of the project files and will maintain the contents of the project files, including all relevant records, reports, field logs, pictures, subcontractor reports, and data reviews in a secured, limited access area and under custody of the AECOM Program Manager.

The project files will contain at a minimum:

- Survey plans and reports
- Station and sample collection log sheets
- Laboratory data deliverables
- Backup QA information
- Data submissions and reports
- All custody documentation (chain of custody forms, air bills, etc.)

Electronic versions of correspondence, reports, and statistical analyses will be stored in the project-specific network file. The original electronic data deliverables (EDD) received from Dr. Moore, and the project data, will also be stored on the AECOM Woods Hole server, which is backed up daily and periodically archived off-site in accordance with AECOM Information Management policy.

Records associated with HOM7 will be retained with all the project records for at least six years after the termination of the project.

B. DATA GENERATION AND ACQUISITION

B1. SAMPLING PROCESS DESIGN (EXPERIMENTAL DESIGN)

A summary of the types and numbers of field samples to be collected during the annual flounder monitoring event is provided in Table 5. The parameters to be measured during the Winter Flounder Monitoring tasks can be characterized as external conditioning, aging of specimens, and histological examination.

B2. SAMPLING METHODS

B2.1 Flounder Collection Procedures

The numbers of field samples and the shipboard processing and storage requirements for all samples to be collected for the flounder monitoring task are listed in Table 5. Dr. Moore (WHOI) will provide supplies needed for shipboard processing of samples.

The flounder monitoring survey will be conducted in late April and/or early May 2010. A commercial otter trawl will be towed by a commercial dragger (*e.g.,* F/V *Harvest Moon*, Captain Mark Carrol), beginning at the center of each station (Table 2). Each tow will last 30–60 minutes at a speed of 1.5 to 2 knots in a direction parallel to lobster-pot sets in the area in order to avoid tangling. Multiple tows will be conducted until at least 50 sexually mature (i.e., >300 mm total length) winter flounder (*Pseudopleuronectes americanus*) have been caught at each of four stations: (1) Deer Island Flats, (2) off Nantasket Beach, (3) offshore outfall site, and (4) eastern Cape Cod Bay (Figure 2).

Navigation data will be obtained from the on-board navigation system for the F/V *Harvest Moon*. Boat position at the start and end of each tow, and depth as determined by a depth sounder, will be recorded by hand onto the field log sheet (Figure 3). Position will be ascertained by GPS, with back-up provided by a ship's LORAN. At all stations, the date and time will also be recorded by hand into the field log sheet.

Using a mechanical winch, the otter trawl will be brought on board and the contents unloaded onto the aft deck of the vessel. All specimens will be sorted to species, but only winter flounder will be retained: all other species will be returned to the sea. Additional tows will be made if 50 acceptable winter flounder are not caught in the first tow. If the required number of flounder is not collected after one 30-minute tow and three 1-hour tows at adjacent sites, collections at that site will be terminated for the survey period. If the number of fish in the first hour of towing is fewer than five (5), the effort will be deferred for two to four weeks.

During the flounder survey, whale observations will be conducted using trained dedicated observers. Whale observations will be documented in the flounder survey summary and the results detailed in the Flounder Survey Report. Field operations for the flounder survey will adhere to the Right Whale Guidance Protocol for Vessels Operated/Contracted by the Commonwealth of Massachusetts. Historical data indicate that there is a relatively high likelihood that right whales will be in Cape Cod Bay while the flounder collections are being made. Dr. Moore is a right whale biologist with field experience since 1979. During transit between stations the Captain, Ms. Winchell (AECOM), and Dr. Moore will maintain a careful lookout for vessels and marine mammals.

Table 5. Parameters, Collection Frequency, Sample Containers, and Preservation Requirements for Flounder Monitoring.

Parameter	Number of Sampling Units Total[a]/Sample[b]	Container	Shipboard or Laboratory Processing/Preservation	Holding Time from Collection
Histology	50/50	Pre-cleaned, labeled polystyrene	Liver tissue processed on boat, preserved in 10% neutral buffered formalin in labeled cassettes	N/A
Histology	50/50	Pre-cleaned, labeled polystyrene	Sample cassettes shipped to EPL, Herndon, VA, still in formalin; EPL prepares histology slides from tissues using standard microtechnique	N/A
Age (scales)	50/50	Age envelope	Shipboard: Clean mucous from sampling area of fish before taking scales Lab: examine under microscope	N/A
Visual	50/50	N/A	Laboratory, Shipboard: Describe qualitatively	N/A
Biometrics • weight • std length • total length • sex	50/50	N/A	Laboratory, Shipboard: Describe quantitatively	N/A

a = total individual specimens collected per station.
b = total number of samples to be analyzed per station.

Station Data for Flounder Survey FF101

Stn #	Station Name	Trawl Date	Start time	Start Depth (m)	Latitude (N)	Longitude (W)	End time	End Depth (m)	Latitude (N)	Longitude (W)	Bottom time (hh:min.)	wf >300 mm	Total fish	time (min)/stn	Fish/min*

Start time is when vessel starts to drag deployed trawl along bottom. End time is when haul back commences.
Only fish counted are winter flounder greater than 300mm in total length

Note last three columns should only have one row per station, irrespective of the total number of trawls at that station.
I.E. sum relevant data from all trawls for each station to generate these parameters.
*Note that Deer Island Fish/min data are calculated separately for the two dates for which that station was occupied

	Available Codes
VESSEL:	
NAVIGATION_CODE: DGPS	DGPS or GPS or LORAN
NAV_QUAL:	±10m and ±30m

VESSEL CREW:

SCIENCE CREW:

Figure 3. Field log sheet for flounder sampling.

B2.2 Flounder Shipboard Processing

Fish processed at sea will be killed by cervical section and processed immediately. Each fish will be assigned a unique sample identification number, which will be generated by Dr. Moore prior to each sampling event. Datasheets and histological cassettes bearing these identification numbers will be prepared in advance to ensure complete collection of both numerical information and samples.

Flounder will be placed blind side up and their gender, weight, and standard and total lengths (Figure 4) will be measured and recorded on the field data sheet (Figure 5). The gross external condition (skin ulcers, lymphocystis, fin rot, bent fin ray, or net damage) will be scored on a scale of 0 to 4 and recorded on the datasheet. Appendix A gives examples of the different types of external lesions that may be seen. Photographs may be taken to document obvious external lesions or ulcers; if such photographs are taken, a notation will be made on the field data sheet. Ten percent of all fish measured at each station will be re-measured for quality control (QC) purposes. Field datasheets will be reviewed after all the fish are processed at a given station in order to assure completeness, accuracy, and legibility.

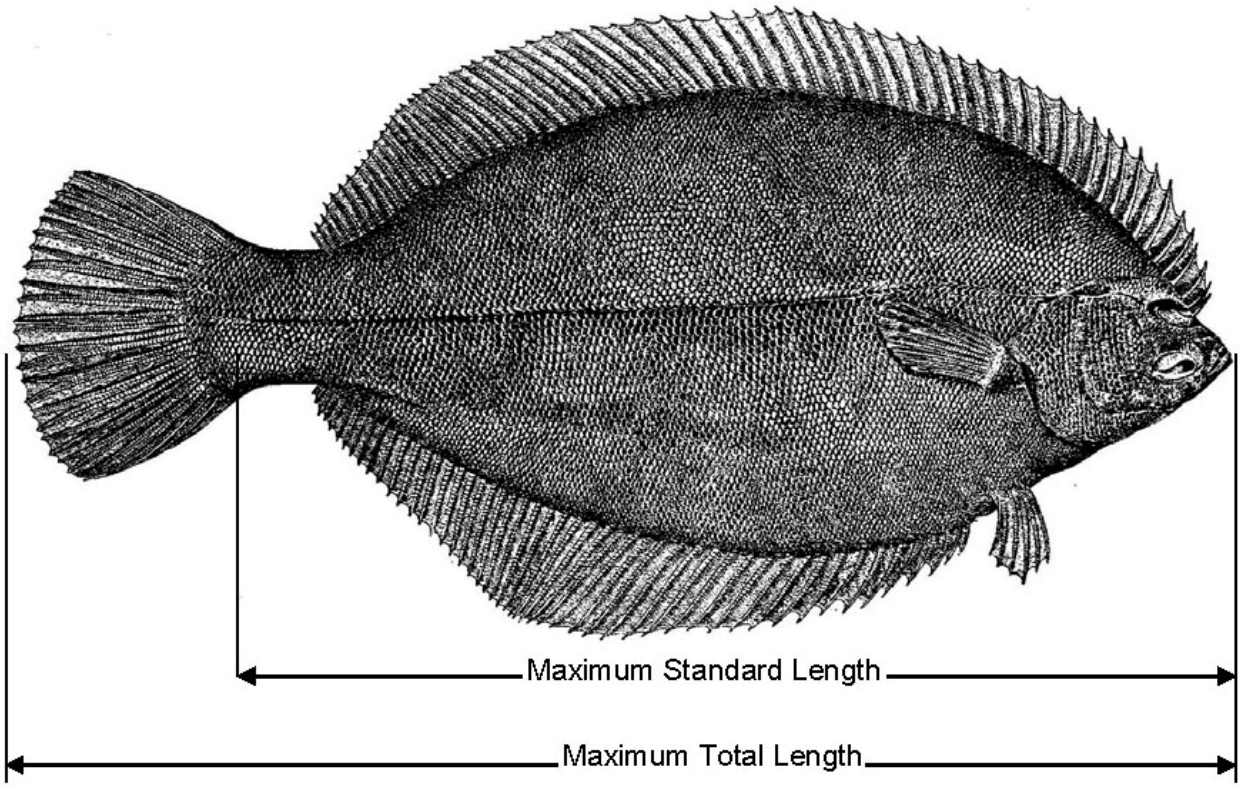

Figure 4. Length measurements for winter flounder, *Pseudopleuronectes americanus*.

2010 External Lesions Data MWRA Harbor and Outfall Monitoring Program Phase 7.

Chain of Custody. Copy completed forms to go with each sample type

Scale_____
Histology_____
Chemistry_____

Relnq_____ __/__/___ Rec'd_____ __/__/___
Rec'd_____ __/__/___
Rec'd_____ __/__/___

Relnq_____ __/__/___
Relnq_____ __/__/___

	ID	Station Name	Weigh t. (g)	TL mm	SL mm	Sex	Age	Fin_ Rot	Bent Fin	Ulce r (0-	Net (0-4)	Lymph o. (0-4)	Liver Color*	Liver Gross+	Photos?
FF101	1001	Deer Island													
FF101	1002	Deer Island													
FF101	1003	Deer Island													
FF101	1004	Deer Island													
FF101	1005	Deer Island													
FF101	1006	Deer Island													
FF101	1007	Deer Island													
FF101	1008	Deer Island													
FF101	1009	Deer Island													
FF101	1010	Deer Island													
FF101	1011	Deer Island													
FF101	1012	Deer Island													
FF101	1013	Deer Island													
FF101	1014	Deer Island													
FF101	1015	Deer Island													

Scientist:_____

Date Collected:____/____/____

*Y:Yellow, YB:Y Brown, B: Brown, DB: Dark Brown

Page __ of __
QA Officer:_____
Date:____/__/____

Figure 5. Form for recording flounder data in the field. Age data will be entered later in the laboratory. This form also serves as the Chain-of-Custody form.

Scales will be collected from each specimen for age determination through the analysis of growth rings (annuli) on the scales. A blunt-edged table knife will be used to remove any mucus, debris, or epidermis from the dorsum of the caudal peduncle by wiping in the direction of the tail. Scales will be removed from the cleaned area by applying quick, firm scraping motions in the direction of the head. Scales will be immediately placed in the pre-labeled age-sample envelope by inserting the knife between the liner of the sample envelope and scraping the scales into the envelope. A temporary check mark will be made on the field data sheet to indicate that this sample has been collected. The samples will be subsequently sent to Mr. Jay Burnett (NMFS) for age determination. After receipt of his data, the results will be entered in the same space, initialed, and dated. These results will be entered into the Access database together with other results on the same data sheet.

All 50 fish collected from each station will be processed for histopathology. Livers will be aseptically removed by severance of the peritoneal attachments and examined for gross abnormalities (gross liver lesions), which will be subjectively scored on a scale of 0 to 4 and recorded on the datasheet (Figure 5). Three slices of liver from each flounder will be placed in a separate, clearly labeled histology cassette. These cassettes will be preserved in a closed bucket of 10% neutral buffered formalin. Other viscera, gonads, heart, and gills will be inspected for gross lesions and recorded on the field data sheet (Figure 5).

B3. SAMPLE HANDLING AND CUSTODY

B3.1 Sample Storage

Storage requirements for flounder samples while in the field are described in Table 5 above. Each sample will be assigned a unique Sample ID, which will consist of the Event ID, the Station #, and a sequential number from 001 through 050 for each individual flounder. The five-character Event ID will be unique to each survey, e.g., “FF101,” where “FF” indicates that it is a flounder survey, “10” indicates the survey year, and “1” signifies the first survey of the year; the stations are 1 = Deer Island, 2 = Nantasket Beach, 4 = Outfall, and 5 = Eastern Cape Cod Bay. Thus, the first fish from Station 1 will be FF1011001 and the 50th fish from Station 5 will be FF1015050. Sample containers are hand labeled by Dr. Moore prior to the field survey.

B3.2 Sample Custody

Sample custody will be tracked through the external lesions data sheet, which will also serve as chain-of-custody forms (Figure 5). Chain-of-Custody (COC) forms will accompany the samples when transferred from the field to the laboratory. All samples will be in the custody of the Chief Scientist, Dr. Moore, who will retain custody upon completion of the survey.

Following each flounder survey, Dr. Michael Moore (WHOI) will personally deliver all histology samples in sealed containers to WHOI and will be responsible for shipping these samples to the EPL in Herndon, VA, for further processing. The samples, which will be preserved in 10% formalin, can be shipped by FedEx Ground or 2-day express delivery. The individually labeled cassettes will be shipped inside a sealed bucket, which will be placed in a sealed cooler. One complete copy of the histology COC forms will be included in each shipping container. Upon arrival at EPL the receiving technician will be responsible for signing the COC forms and the originals will be returned to WHOI.

While at EPL, the tissue slices will be embedded in the same tissue cassettes that were labeled at the time of collection. The Sample ID number will be copied from the cassettes to the slides at the time of sectioning the embedded blocks. Once the samples have been processed by EPL, the blocks and slides will be returned under COC to Dr. Moore at WHOI via FedEx.

The fish scales used for aging the flounder will be placed in individual envelopes during shipboard processing and will be hand delivered under COC to the NMFS building in Woods Hole, MA, by Dr. Moore.

A copy of the COC form will be retained in the field log. Copies of the COC form will be made as needed and will accompany the samples to the laboratory for subsequent sample transfer. When samples arrive at each of the laboratories, custody will be relinquished to the laboratory custodian, who will examine the samples, verify that sample-specific information recorded on the COC is accurate and that the sample integrity is uncompromised, log the samples into the laboratory tracking system, and complete and sign the COC form so that transfer of custody of the samples is complete. Any discrepancies between sample labels and transmittal forms, and unusual events or deviations from the QAPP will be documented in detail on the COC and the Project Area Manager for MWRA and Program Manager for AECOM will be notified within 24 hours. Copies of completed custody forms will be faxed back to the AECOM task manager within 24 hours of receipt. The signed original custody forms will be retained in Dr. Moore's project files.

B3.3 Sample Archival Policy

The types of materials that may be archived under this Flounder Monitoring Task include slides containing liver sections and scales used in aging. The slides will be archived by Dr. Moore and held within the WHOI facilities until the data report is finalized by MWRA; they may then be discarded. The scales used for aging the fish at NMFS will be archived at the NMFS facilities until the data report is finalized by MWRA, and then may be discarded.

B4. ANALYTICAL METHODS

B4.1 Histological analysis

Flounder liver tissue will be sectioned and slides prepared at EPL in Herndon, Virginia. The histology cassettes containing the liver sections from each flounder will be placed in a sealed 5-gallon shipping container and shipped by Dr. Moore via FedEx Ground or 2-day express delivery to EPL in Herndon, VA. The samples will be shipped in 10% neutral buffered formalin and will remain in the buffered formalin for at least 24 hours, and then removed, rinsed in running tap water, dehydrated through a series of ethanols, cleared in toluene or 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, with one slide per flounder and three replicate liver slices per slide, for a total of 200 slides and 600 replicates per year. The sections will then be stained in Hematoxylin and counter-stained in Eosin. The slides will be shipped back to Dr. Moore at WHOI for analysis.

Dr. Moore will use the same methods for histological examination of the slides that he has used in previous years. Utilizing previously established criteria for this project, each liver histology slide will be examined by Dr. Moore under bright-field illumination at 25×, 100×, and 200× to quantify the presence and extent of:

- Vacuolation, seen in three stages:
    - Centrotubular hydropic vacuolation: isolated groups of 1−2 vacuolated cells in the center of the hepatic tubule.
    - Tubular hydropic vacuolation: linear arrays of vacuolated cells, filling the hepatic tubule, often extending into biliary duct structures.
    - Focal hydropic vacuolation: foci of 30 to several hundred contiguous vacuolated cells.
- Macrophage aggregation - circular golden brown cellular masses often associated with fibrotic tracts, bile ducts, and blood vessels.
- Biliary duct proliferation - branching ducts often ensheathed by fibrosis.
- Neoplasia - focal, often grossly visible areas of cells fulfilling established criteria for neoplasia in this species.
- Other lesions, such as apoptotic lesions (*i.e.*, balloon cells).

The severity of each of the above lesions will be scored on a scale of 0 to 4, where 0 = absent, 1 = minor, 2 = moderate, 3 = severe, and 4 = extreme.

In addition, the presence of liver flukes will be recorded as they have been since 2008. They will be scored as follows: 0 absent, 1 rare, 2 common and 3 abundant.

B4.2 Age Determination

Age determination performed through the analysis of growth rings (annuli) on the scales collected from each flounder will be conducted by Dr. Jay Burnett at the Age and Growth Unit of the NMFS (Fields, 1988).

B5. QUALITY CONTROL

B5.1 Sampling

B5.1.1 *Navigation*

Navigation will be performed using the navigation system of the F/V *Harvest Moon*. All navigation information will be recorded by hand onto field log sheets (Figure 4).

Accuracy and Precision
The onboard navigation system uses the differential GPS (dGPS) for station positioning. The reporting units for the dGPS navigation are degrees, the range is coastal, and accuracy and precision of both are 1.8×10^{-5} degrees.

Comparability
All sampling positions will be comparable to positions obtained by previous MWRA flounder monitoring activities as well as by other researchers that have used or are using dGPS at these stations. The station locations listed in Table 2 are targets, and at each sampling station the vessel is positioned in the general vicinity of the target position to begin the trawl. Each trawl is of 30-minute or 1-hour duration with the boat often changing course due to bottom anomalies noted on the boat's fathometer.

Completeness
Navigation information will be recorded by hand. Entries for each station will be reviewed by the Chief Scientist before leaving each station to ensure that all information has been entered onto the field data sheets.

B5.1.2 *Flounder Collection and Shipboard Processing*

All flounder will be collected with a commercial otter trawl. Only those specimens of winter flounder (*Pseudopleuronectes* americanus) meeting project criteria (see section B2.1) will be retained. All other species and specimens not meeting requirements will be returned to the environment.

Accuracy
Traditional measures of accuracy do not apply directly to fish collection procedures. Accuracy measures do apply to the identification, measurement, and weighing of fish.

To ensure that specimens are accurately identified, taxonomic keys and various field guides will be used. The guaranteed accuracy of the Ohaus fish scale is 20g. The accuracy of the fish measuring board is 0.1cm.

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. Data Quality Objectives (DQOs) for precision of physical measurements are defined in Table 6, along with corrective actions.

Table 6. Data Quality Objectives (DQOs) for Physical Measurements of Flounder

QC Type and Frequency	Acceptance Criteria	Corrective Action
Precision Duplicate Measurements 10%	Flounder weight: ± 20 grams Flounder Total and Standard Length: ± 1 cm	Check calibration of balance, if applicable. Review procedures if length is wrong

Completeness
The objective is to obtain 50 sexually mature specimens from each of four sampling sites. 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., resampling using a different otter trawl). The corrective action taken by the Chief Scientist will be recorded in the field logs. If the required number of flounder is not collected after one 30-minute and three 1-hour tows at an appropriate adjacent site, collections at that site will be terminated for the survey period. The MWRA Project Manager will be notified of these circumstances and may accept the survey as 100% complete. If the number of fish in the first hour of towing is less than five (5), the effort will be deferred for two to four weeks. All flounder will be weighed and measured, and the recorded datasheet will be checked for completeness by the Chief Scientist prior to processing the specimens.

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. Moore for previous studies on winter flounder from these sampling sites. Otter trawls have been used by the National Oceanic and Atmospheric Administration (NOAA) NMFS and the Massachusetts Division of Marine Fisheries (DMF) since the 1960s and 1970s, respectively, as a method to sample finfish. All otter-trawl tows will be conducted for approximately 30 to 60 minutes at a speed of 1.5 to 2 knots. The sampling design of this survey is comparable to the design of previous surveys.

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. Representativeness will also be ensured by proper handling, storage, and analysis of samples, as defined in this QAPP, so that the flounder tissues collected and analyzed reflect the conditions at the site locations.

B5.2 Flounder Histological Analysis and Age Determination

Accuracy

Traditional measures of accuracy do not apply to the flounder histological analyses. 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. Histological observations will be made by Dr. Moore, a highly trained scientist with decades of experience in evaluating flounder tissue abnormalities. An inter-comparability exercise carried out in 1992 documented that two 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 showed 100% agreement with earlier results. 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.

Flounder scales will be read by NMFS scientists who are experienced in aging winter flounder.

Precision

The precision of the accuracy of age determination will be monitored through re-reading 10% of the scales. See the section above on Accuracy for a discussion relating to the accuracy and precision of histological evaluations made in this monitoring program.

Completeness

Tissues and scales from all 50 flounder collected from each station during the survey will be examined for evidence of disease and age determination, respectively. The datasheet used to record the information will be checked for completeness. Lesion scores will be calculated using three slides of liver tissue from each of 50 flounder collected at each site, thus providing sufficient data to perform the statistical analyses needed to assess the health of flounder populations, as well as to make inter-site comparisons of lesion prevalence.

Comparability

The lesions scored, the method of scoring, and the data reduction and analyses will be similar to procedures used in previous years for the HOM program. Dr. Moore has been performing the histological analyses and the NMFS scientists have been aging the scales for many years as part of this program. Comparability of flounder liver histology data has been confirmed in a number of studies described in the section on Precision (above).

Representativeness

The program design and objectives ensure representativeness. Representativeness will also be ensured by proper handling and preparation of the liver samples for histology analysis, as defined in this QAPP, so that the flounder tissues collected and evaluated reflect conditions at the site locations.

B6. INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE

Maintenance of and repairs to instruments will be in accordance with manufacturers' manuals.

B6.1 Field Equipment Maintenance Requirements

Equipment will be monitored 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. 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.
- Otter trawls will be checked by the Captain after each trawl for possible damage.

B6.2 Laboratory Equipment

The primary analytical instrument used for the histological analyses is a Zeiss photomicroscope. 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. Similar care will be applied to the stereomicroscope used by Mr. Burnett at NMFS for the age analysis task.

Under HOM7, there are no analytical laboratory instruments covered by this QAPP.

B7. INSTRUMENT/EQUIPMENT CALIBRATION AND FREQUENCY

B7.1 Navigation Equipment

Typically, the Captain of the boat being used will provide the GPS navigation system and will ensure that the instrumentation is maintained and calibrated. However, because the primary business of the boats used for this project is commercial fishing and not scientific collection, maintenance schedules are irregular. Therefore, Dr. Moore uses a portable Garmin GPS12 unit as a back-up to compare with the boat's output or to use in lieu of the boat GPS unit. Dr. Moore has his unit calibrated prior to the annual survey.

B7.2 Laboratory Equipment

Field equipment to be calibrated includes only the scale used to weigh the fish. The Ohaus® dial scale, Model No.8014 MA, will be calibrated with a known weight after sampling has been completed at each sampling site (i.e., prior to use at the next sampling site). The scale will be inspected prior to measuring each fish to ensure that it reads zero.

The primary analytical instrument used for the histological analyses is a Zeiss photomicroscope. No calibrations are required.

EPL will calibrate the microtome prior to cutting thin sections to ensure it is set to cut at the required thickness.

Under HOM7, there are no analytical laboratory instruments covered by this QAPP.

B8. INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES

For HOM7, critical supplies for field activities will be the responsibility of the Chief Scientist, Dr. Moore, and the boat Captain (Table 7).

Table 7. Supplies, Acceptance Criteria, and Responsibility for Critical Field Supplies.

Critical Supplies and Consumables	Inspection Requirements and Acceptance Criteria	Responsible Individual
Histology cassettes for samples	Visually inspected for cracks, breakage, and cleanliness.	Chief Scientist
Envelopes for scale samples	Visually inspected for tears	Chief Scientist
Chemicals and reagents	Visually inspected for proper labeling, expiration dates, appropriate grade.	Chief Scientist
Sampling equipment	Visually inspected for obvious defects, damage, or contamination.	Chief Scientist and Boat Captain
Navigation instruments	Functional checks to ensure proper calibration and operating capacity.	Chief Scientist and Boat Captain

If unacceptable supplies or consumables are found, then the Chief Scientist will initiate corrective action. Corrective measures may include repair or replacement of measurement equipment, and/or notification to vendor and subsequent replacement of defective or inappropriate materials. All actions will be documented in the project files.

B9. NONDIRECT MEASUREMENTS

Nondirect data (historical reports, maps, literature searches, and previously collected analytical data) may be used in the preparation of the flounder annual reports (Task 11). These data may come from sources such as:

- Prior MWRA harbor and outfall monitoring program results
- Pertinent data collected by other agencies, such as NMFS, MA DMF

B10. DATA MANAGEMENT

Figure 6 illustrates the strategy for processing data generated under the flounder monitoring task including data entry into the MWRA EM&MS database and accessing the data for reports. Data from the program will be compared by MWRA to the caution and warning threshold parameters (Table 3) included in the MWRA Contingency Plan (MWRA 2001).

B10.1 Data Custody

Field custody of data will be the responsibility of the Chief Scientist, Dr. Moore. After the survey, flounder survey data entered on field logs will be keyed by Dr. Moore into an Excel spreadsheet provided by Battelle. This spreadsheet will be suitable for loading into an Access loading application. Verification will be provided through a 100% quality check of the electronic data against the field logs by Ms. Stacy Doner (AECOM). This Access file will then be delivered by AECOM to Mr. Greg Lescarbeau at Battelle for processing. A csv file will be generated by Battelle for AECOM that will be acceptable for loading by MWRA according to their rules. The csv file will be reviewed by Ms. Doner to ensure that all required fields have been properly converted from the Access application. Dr. Blake will then submit the file electronically to the MWRA Project Manager. After loading the data, MWRA will produce a field data table that will be provided to AECOM for inclusion in the Survey Report**.**

Dr. Moore will enter data from the histological and scale (age) analyses directly into the loading application provided by Battelle, and will then forward the file to the AECOM laboratory task manager, Ms. Stacy Doner. After Ms. Doner checks the data package for completeness, the loading application will be delivered to Mr. Greg Lescarbeau at Battelle for processing. A csv file will be generated by Battelle for AECOM that will be acceptable for loading by into the MWRA EM&MS database as described in section B10.3 below.

Data to be used in the annual reports must be requested from MWRA, who will generate a data export from the EM&MS database.

B10.2 Laboratory Data and Data Reduction

All data generated by WHOI will be manually read from the optical field of a microscope and entered directly into the Access database format provided by Battelle. WHOI will submit the quality checked, electronic data to the AECOM data manager, Ms. Stacy Doner. AECOM will use the LeapFILE secure file exchange provider for transferring EDDs between AECOM and MWRA and other team members when relevant. Recipients will be notified of submittal by email. Files will be available on the LeapFILE site for transfer for 14 days, after which time they are removed from the site. The sender is notified when the recipient opens the email notification, and when the recipient successfully downloads the files. This method of tracking the electronic file transfers allows the sender to remind recipients that the data is ready for pick-up if necessary, prior to the deletion of the file. Once the HOML application is available, electronic data submissions will be made using that web application.

Data reduction is the process of converting raw numbers into data that can be displayed graphically, summarized in tables, or compared statistically for differences between mean values for sampling times or stations. Data reduction may take place (1) before submission to MWRA and/or (2) before inclusion in the annual report. Few data generated by the Flounder Monitoring task require any manipulation before being submitted to the EM&MS. All data reduction for report preparation will be performed electronically (e.g., in Excel) and will be validated according to procedures described in Section D2.

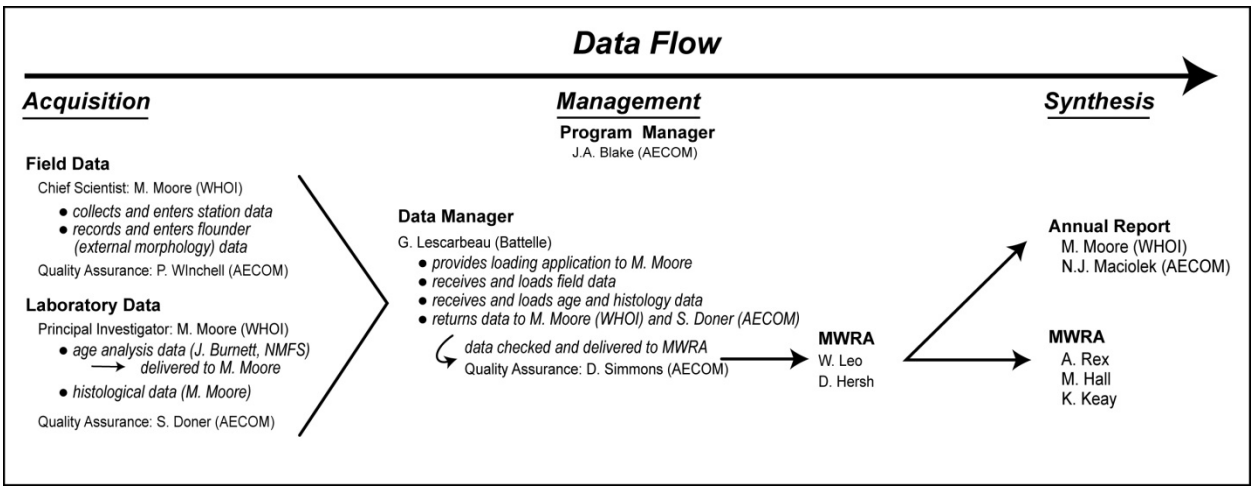

Figure 6. Data management for flounder monitoring task.

B10.2.1 *Flounder Survey Data*

No data reduction is performed on navigation data. All sample collection information will be recorded by hand and transferred to an electronic format at WHOI. The Catch Per Unit Effort (CPUE, i.e., 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. Bottom depth values will be recorded at the start and end of each trawl.

B10.2.2 *Histology Data*

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. Data resulting from the assignment of scores to the various lesions will be transferred in electronic format, first to project database personnel and then to MWRA (see section B10.3)

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.

B10.3 Analytical Data Sets Submitted for Loading into the MWRA Database

All laboratory data acquired under the Flounder Monitoring Tasks to be loaded into the EM&MS will be submitted by AECOM in electronic format. Battelle will provide a loading application to Dr. Moore at WHOI, where he will enter the laboratory data. The completed loading application will be sent to Battelle, where an Excel spreadsheet will be prepared. The spreadsheet will be reviewed by Dr. Moore and AECOM to ensure that it is correct and complete. If the spreadsheet is approved, Battelle will generate a csv file for submission to MWRA and will provide this file to AECOM for final submission to MWRA.

Formats for delivering electronic data to MWRA are included in the HOM7 contract but these formats are subject to change. The current delivery formats are available from the data task managers at Battelle (Greg Lescarbeau) or at MWRA (Wendy Leo). Battelle's data management staff will process all data into the appropriate HOML format as defined in the contract and will deliver these data to AECOM for review. These submissions then will be delivered by AECOM to MWRA via email in the absence of the HOML application. Once the HOML application goes online, AECOM will submit data electronically through the application.

B10.3.1 *Navigation and Sample Collection Data*

Navigation and sample collection data will be processed by WHOI and delivered to the Battelle data manager as an Excel spreadsheet. Battelle's data management staff will process all data into the appropriate HOML format as defined in the contract and will deliver these data to AECOM for review. After verification that the file is complete, the data will be submitted to EM&MS. All database constraints developed by MWRA will be applied to the tables so that the data are checked during insertion.

B10.3.2 *Laboratory Analytical Data*

Battelle will send a loading application specific to the flounder histological and age analyses to Dr. Moore at WHOI, where the laboratory data will be entered (Figure 8). When the data entry screen is opened, he will be presented with a form that is already populated with sample identification numbers (Figure 8B). All data entries are constrained by the rules of EM&MS. Errors are caught on entry and fixed by the data contributor. Primary keys are in place so duplication cannot occur.

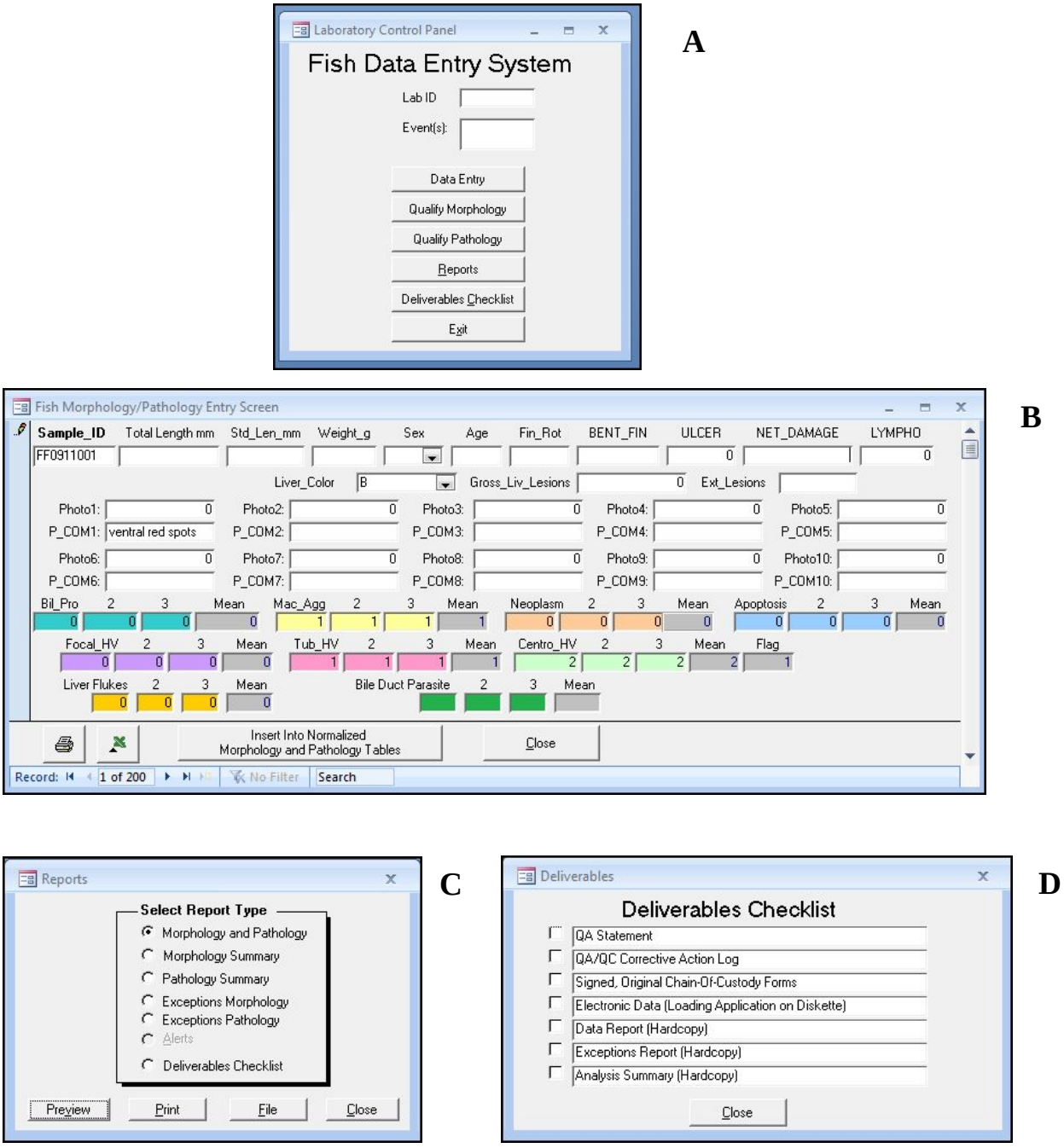

Figure 8. Loading application for flounder data. (A) Initial screen. (B) Data entry screen. (C) Reports options. (D) Deliverables options.

B10.3.3 *Loading Application Functions and Database Codes*

The loading application provides the laboratory many available functions, including quality control checks, hardcopy and exceptions reports, and analysis summary (Figure 8C, D). The hardcopy data report function allows the laboratory to create a hardcopy report to check for entry errors and to include as part of the data deliverable. The quality control checks are comprised of the applicable sections of EM&MS and constraints scripts. The exceptions report checks the data that were expected against the results loaded. The data contributor must account for any entries in the exception report. A copy of this report is included with the data deliverable to MWRA and with the invoice for the analyses.

Within the loading application, the data entered by the laboratory are translated into the correct codes and inserted into database tables that match the structure used by EM&MS. It is anticipated that there will be limited need to establish new codes for the flounder database. Observation of previously unseen abnormalities on flounder could require new codes. If this or any other unforeseen situations arise during the course of the project, Battelle will create a provisional code so that data can continue to be recorded until the new code can be submitted to and approved by MWRA. Any new qualifiers will be highlighted in the exceptions report. Battelle will notify AECOM and 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 has the responsibility for maintaining the code list for the EM&MS. The current codes are listed in Tables 8 through 11.

Table 8. Data Qualifiers

Qualifier	Description	Value Reported?
	Value is not qualified	Yes
A	Value above maximum detection limit, e.g. too numerous to count or beyond range of instrument	Yes
a	Not detected – value reported as negative or null	Yes
aq	Not detected - value reported as negative or null. May be invalid, under investigation (Do not use).	No
as	Not detected - value reported as negative or null, and not fit for use	No
e	Results not reported, value given is NULL, see comments field	No
eq	Not reported, may be invalid, under investigation (Do not use)	No
q	Possibly suspect/invalid and not fit for use. Investigation pending.	Yes
s	Suspect/Invalid. Not fit for use	Yes
w	This datum should be used with caution, see comment field	Yes

Table 9. Morphological Parameters and Database Codes for Flounder Monitoring.

Parameter Description	Param_code	Meth_Code	Unit_code
Chronological age of specimen	AGE	SCALE	y
Gender	SEX	VISUAL	
Standard length of fish – from upper jaw tip to posterior end of the hypural bone	SLM	VISUAL	mm
Total Length	TLM	BSOP5-175	mm
Wet weight of organism	WEIGHT	PWEIGHT	g

Table 10. Histopathological Parameters and Database Codes for Flounder Monitoring.

Parameter Description	Param_code	Fraction Code	Meth_code
Apoptotic lesion prevalence, rated on a scale from 0-4	BALLOONS	LIVER_SECTION	FSF98
Biliary proliferation	BIL_PROLIF	LIVER_SECTION	FSF98
Centrotubular hydropic vacuolation	CENTRO_HV	LIVER_SECTION	FSF98
Liver flukes	LIVER_FLUKES	LIVER_SECTION	FSF98
Focal hydropic vacuolation	FOCAL_HV	LIVER_SECTION	FSF98
Macrophage aggregation, rated on a scale from 0-4	MACROPHAGE	LIVER_SECTION	FSF98
Neoplasia prevalence, rated on a scale from 0-4	NEOPLASM	LIVER_SECTION	FSF98
Presence (1) or absence (0) of flounder liver bile duct unicellular parasites in histology sections	BILE_DUCT_PROTOZOAN	LIVER_SECTION	FSF98
Tubular hydropic vacuolation	TUBULAR_HV	LIVER_SECTION	FSF98
Fin rot score	FIN_ROT	WHOLE_BODY	FSF98
Gross lesions visible on whole flounder liver	GROSS_LIV_LESIONS	WHOLE_BODY	FSF98
Flounder skin ulcer	ULCER	WHOLE_BODY	FSF98
Lymphocystis	LYMPHO	WHOLE_BODY	FSF98
Bent fin ray	BENT_FIN	WHOLE_BODY	FSF98
Net damage	NET_DAMAGE	WHOLE_BODY	FSF98
Liver color	LIVER_COL	WHOLE_BODY	FSF98

Table 11. General Database Codes for Flounder Monitoring.

Field Name	Code	Description
ANAL_LAB_ID	WHO4	Woods Hole Oceanographic Institution – M. Moore
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
INSTR_CODE	MICR	Microscope
INSTR_CODE	RULER	Measurement by ruler
MATRIX_CODE	8857041504	*Pseudopleuronectes americanus*
METH_CODE	EYE	Visual inspection
METH_CODE	FSF98	Method for pathology parameters described in fish and shellfish QAPP
METH_CODE	PWEIGHT	Flounder weight measurement mentioned in QAPP for Flounder Monitoring Sec. B.2.2 (AECOM 2008)
METH_CODE	SCALE	Aging by scale
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 QAPP for Flounder Monitoring Sec. B.2.2 (AECOM 2008)
QC_CODE	SAMP	Normal sample
UNIT_CODE	g	grams
UNIT_CODE	mm	millimeters

B10.4 Loading Analytical Data into the Harbor and Outfall Studies Database

Data submissions from WHOI will consist of a final loading application as discussed above. The submissions will be logged in upon receipt at Battelle and a copy of the login will be maintained on file under the login id. Data will be loaded into a temporary table space by striking a button on the application. A transfer script will copy the data into the proper table in Battelle's copy of the HOML. Data will receive a quality assurance review by Battelle prior to electronic submission to AECOM. Any issues will be corrected in the database and the script output will be supplied to MWRA and AECOM upon request. A check script will be run on the database prior to export of a dataset to ensure that all data conform to quality control checks and database constraints. Any issues will be sent to the Battelle Data Manager and AECOM Program Manager via e-mail. 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.

B10.5 Data Report Quality Control Checks

Range checks will be performed on the parameters given in Table 12. These checks will be done by MWRA and reviewed by AECOM and WHOI as part of the data reporting process (see section A.9.5).

Table 12. Range Checks for Flounder Monitoring.

Parameter	Action
Length	Range check against longest and shortest flounder from previously acceptable data. Specimens outside this range will be flagged.
Weight	Range check against previously acceptable data. Specimens outside this range will be flagged.
Age	Tabulate age vs. length and weight. Outliers will be flagged, and the measurement reevaluated.
External Abnormalities	0-4 range check for each abnormality. Specimens outside this range will be flagged.
Liver Histopathology	0-4 range check for each histopathology parameter. Specimens outside this range will be flagged. Plot prevalence by station to ensure that no obvious errors, such as reporting tumors at a station where none were seen.

C. ASSESSMENT AND OVERSIGHT

C1. ASSESSMENT AND RESPONSE ACTIONS

This section identifies the number, frequency, and type of planned assessment activities that will be performed to assure implementation of this QAPP for HOM7 flounder monitoring. These activities will be overseen by the AECOM Project QA Officer, Ms. Debra Simmons.

C1.1 Assessments

C1.1.1 *Field Sampling Readiness Reviews*

Each field survey plan will include a checklist for required supplies and equipment, including items required for health and safety of field personnel. An example is shown in Table 13.

C1.1.2 *Field Sampling Technical System Audit*

The Project QA Officer and/or Chief Scientist will be responsible for periodic internal Technical Surveillance Audits (TSAs) to verify that field sampling procedures and measurements are properly followed. If the subcontractor does not have the capability to perform internal auditing, an audit will be conducted by AECOM at project start up and then periodically as part of its analytical subcontractor monitoring program. The field audit may consist of an actual visit to the boat during mobilization or a review of procedures at the time the Survey Plan is developed. The internal field audit checklist (Table 14) will include examination of the following:

- Field sampling records
- Sample collection, handling, and packaging procedures
- QA procedures
- Chain-of-custody
- Sample documentation

Preliminary results of the systems audit will be discussed with the Chief Scientist, or with the field team, as appropriate. A written report that summarizes audit findings and recommends corrective actions will be prepared and submitted to the AECOM Program Manager. The results of the audit, including resolution of any deficiencies, will be included in the QA reports to management, as described in Section C2.

Table 13. Flounder Survey Supply Checklist

FIELD SAFETY AND EQUIPMENT CHECKLIST

FIELD SAFETY CHECKLIST

Date of Survey_____

Project No. _____

Type of work:
- Sample collecting
  - Landbased ☐
  - Waterbased ☐
- Mooring operations ☐
- Dive operations ☐
- Towed sampling ☐
- Navigation ☐
- Other:_____ ☐

Type of sample collected:
- Water ☐
- Sediment ☐
- Sludge ☐
- Raw sewerage ☐
- Dredge materials ☐
- Living organisms ☐
- Marine debris ☐
- Electronic data ☐
- Other:_____

	Y	N
*Do samples impose a health risk?	☐	☐
If yes, what kind of hazard:		
Chemical	☐	☐
Biological	☐	☐
Radioactive	☐	☐
Other _____		
Specify Hazard:_____		
* (or fixatives / additives used w/ samples)		
Is there a spill response plan?	☐	☐
Is one necessary?	☐	☐
Are immunizations necessary?	☐	☐
Will electrical equipment be used by staff?	☐	☐
Will electrical equipment be used on deck?	☐	☐
Will ground fault interrupt (GFI) be used?	☐	☐
Will elecrtrical equipment be checked-out before survey?	☐	☐

List type of sampling equipment to be used:
_____

Do all members of the survey party have appropriate field experience?	☐	☐
Is training necessary before the survey?	☐	☐
Will there be lifting of heavy objects?	☐	☐
Are all members of survey party familiar with safe lifting practices?	☐	☐

Reviewed and approved

Task Leader_____ Date_____

Chief Scientist_____ Date_____

Dept Manager_____ Date_____

FIELD SAFETY EQUIPMENT CHECKLIST

Check equipment needed for survey

	Tech Staff	Lab Staff
Hard Hats**		
Work Vests**		
Life Raft		
EPIRB		
First Aid Kit		
Cold Weather Suits		
Safety Glasses		
Work Gloves		
Tyvek Suits		
Radiation Detector		
Respirators		
Air Hood		
Face Shields		
Lab Coats		
Eye Wash		
Flash Lights		
Spill Response Kit		

** Required for surveys using vessels

Survey Party, Battelle Staff	ENSR Staff
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

Table 14. Example of Internal Field TSA Checklist

Project: **Site Location:** **Auditor:**	
1. Was project-specific training held?	
2. Are copies of project plan (SAP, QAPP) on site and available to personnel?	
3. Are samples being collected in accordance with the project plan?	
4. Do the numbers and locations of samples conform to the project plan?	
5. Are sample locations staked or otherwise marked?	
6. Are samples labeled in accordance with the project plan?	
7. Is equipment decontamination in accordance with the project plan?	
8. Is field instrumentation being operated and calibrated in accordance with the project plan?	
9. Are samples being preserved and containerized in accordance with the project plan?	
10. Are QC samples in accordance with the types, collection procedures, and frequencies specified in the project plan?	
11. Are chain-of-custody procedures and documents in conformance with the project plan?	
12. Are field records complete, accurate, up-to-date, and in conformance to good recordkeeping procedures?	
13. Are modifications to the project plan being communicated, approved, and documented appropriately?	
Additional Comments:	
Auditor:	**Date:**

C1.1.3 *Fixed Laboratory Technical System Audits*

System audits will be performed as described in subcontractor's QA manual. If the subcontractor does not have the capability to perform internal auditing, an audit will be conducted by AECOM at project start up and then periodically as part of its analytical subcontractor monitoring program. The laboratory audit will consist of either an on-site visit to the facility or a telephone interview. The audit will consist of a review of the following, as appropriate for the type of audit being conducted (an example checklist is included in Table 15):

- QA organization and procedures
- Personnel training and qualifications
- Sample log-in procedures
- Sample storage facilities
- Analyst technique
- Adherence to laboratory SOPs and project QAPP
- Compliance with QA/QC objectives
- Instrument calibration and maintenance
- Facility security
- Waste management
- Data recording, reduction, review, reports, and archival
- Cleanliness and housekeeping

Preliminary results of the systems audit will be discussed with the laboratory point-of-contact (see Table 1). A written report that summarizes audit findings and recommends corrective actions will be prepared and submitted to the laboratory point-of-contact for response and to the AECOM Program Manager. The results of the audit, including resolution of any deficiencies, will be included in the QA reports to management, as described in Section C2.

C1.1.4 *Performance Evaluation Sample Assessment*

Proficiency testing for histological analyses has been accomplished through regular communication and intercalibration of histology samples among laboratories.

C1.1.5 *Data Technical System Audits*

Tabular data reported in deliverables and associated raw data generated by WHOI will be audited by AECOM personnel under the direction of the AECOM Project QA Officer for 100% of the packages received as part of the data validation process (Section D.1). Raw data will be reviewed for completeness and proper documentation. Errors noted in data audits will be communicated to analysts and project management and corrected data will be verified. Audits of the data collection procedures at subcontractor laboratories will be the responsibility of the subcontractor laboratories. Each subcontractor is fully responsible for the verification and validation of the data they submit. Data must be submitted in QAPP-prescribed formats; no other formats will be acceptable. During the time that work is in progress, the subcontractor QA Officer or his/her designee will conduct an inspection to evaluate the laboratory data-production process. All data must be reviewed by the subcontractor QA Officer or an individual other than the person responsible for data generation prior to submission to Ms. Stacy Doner, the AECOM Data Task Manager, and must be accompanied by a signed QA statement that describes the types of audits and reviews conducted, the results, any outstanding issues that could affect data quality, and a narrative of activities.

Table 15. Example of Laboratory Audit Checklist

Project: **Facility Location:** **Auditor:**	
Is there a written QA Program Plan/Manual?	
Is there a designated QA Officer?	
Are facilities and equipment adequate to perform the analyses of interest?	
Review procedures and engineering controls for minimizing cross contamination.	
Review most recent inter-laboratory performance evaluation sample results and recent Agency audits.	
Review SOP system. Review techniques for conformance to approved SOPs.	
Are personnel qualified and trained? Is there a formal training program and are records of training and proficiency maintained?	
Is there a designated sample custodian? Is there a sample inspection checklist? Are sample log-in procedures defined in an SOP?	
Is the laboratory area secure?	
Review internal chain-of-custody procedures.	
Are instruments operated and calibrated in accordance with SOPs? Are records of calibration maintained?	
Is equipment maintained according to written protocols? Are routine and non-routine maintenance procedures documented?	
Are samples being analyzed in conformance to the cited methods?	
Are QC samples and checks being performed at the frequencies stated in the cited methods?	
Are records complete, accurate, up-to-date, and in conformance to good recordkeeping procedures?	
How are project-specific requirements communicated to the bench level?	
Review data reduction, review, and reporting processes.	
Review data archival process (paper and electronic).	
Review audit and corrective action program.	
Additional Comments:	
Auditor:	**Date:**

C1.2 Assessment Findings and Corrective Action Responses

All technical personnel share responsibility for identifying and resolving problems encountered in the routine performance of their duties. Issues that affect the schedule, cost, or performance of tasks associated with the flounder monitoring will be reported to Dr. James A. Blake, AECOM's Program Manager. He will be accountable to MWRA and to AECOM management for overall conduct of the Harbor and Outfall Flounder Monitoring Project, including the schedule, costs, and technical performance. Dr. Blake will be responsible for identifying and resolving problems that (1) have not been addressed in a timely manner or successfully at a lower level, (2) influence multiple components of the project, or (3) require consultation with AECOM management or with MWRA. He will be responsible for evaluating the overall impact of the problem on the project and for discussing corrective actions with Dr. Moore and the MWRA Flounder Monitoring Project Area Manager. He will also identify and resolve problems that necessitate changes to this QAPP. Problems identified by the AECOM Project QA Officer will be reported to him and corrected as described in Section C2.

Corrective action is the process of identifying, recommending, approving, and implementing measures to counter unacceptable procedures or out-of-limit QC performance that can affect data quality. Corrective action can occur during field activities, laboratory analyses, data validation, and data assessment. All corrective action proposed and implemented should be documented in the QA reports to management (Section C2). Corrective action should only be implemented after approval by the appropriate personnel (as identified in the following sections).

C1.2.1 *Field Corrective Action*

Corrective action in the field may be needed when the sample frequency is changed (*i.e.*, more/fewer samples, sample locations other than those specified in this QAPP), or when sampling procedures and/or field analytical procedures require modification due to unexpected conditions. The field team may identify the need for corrective action. The MWRA Flounder Monitoring Project Area Manager, AECOM Program Manager, and AECOM Project QA Officer will approve the corrective measure. The Chief Scientist will ensure that the field team implements the corrective action.

Corrective action resulting from internal field audits will be implemented immediately if data may be adversely affected due to unapproved or improper use of approved methods. The QA auditor will identify deficiencies and recommend corrective action to the Chief Scientist. The Chief Scientist and field team will perform implementation of corrective actions. Corrective action will be documented in QA reports to the project management team (Section C2).

Corrective actions will be implemented and documented as follows in the field records:

- A description of the circumstances that initiated the corrective action
- The action taken in response
- The final resolution
- Any necessary approvals
- Effectiveness of corrective action

No staff member will initiate corrective action without prior communication of findings through the proper channels. If at any time a corrective action issue which directly impacts the project DQOs is identified, the MWRA Flounder Monitoring Project Area Manager will be notified.

C1.2.2 *Laboratory Corrective Action*

Corrective action in the laboratory may occur prior to, during, and after initial analyses. Conditions, such as broken or missing sample containers, discrepancies between the samples received and COC paperwork, and/or sample loss or breakage, may be identified during sample log-in or analysis. Following consultation with laboratory personnel, it may be necessary for the subcontractor point of contact to approve the implementation of a corrective action. If the problem makes it impossible to achieve project objectives, the AECOM Laboratory Task Manager will be notified, who will in turn notify the AECOM Program Manager and AECOM Project QA Officer. The AECOM Program Manager will communicate with the MWRA Flounder Monitoring Project Area Manager and other members of the project team, as necessary. The MWRA Flounder Monitoring Project Area Manager will also be notified in those cases where the nonconformance affects the achievement of the project DQOs.

These corrective actions will be performed prior to release of the data from the laboratory. The corrective action will be documented in both the laboratory's corrective action files and the narrative data report. If the corrective action does not rectify the situation, the laboratory will contact the AECOM Laboratory Task Manager, who will determine the action to be taken and inform the appropriate personnel.

C1.2.3 *Corrective Action during Data Validation and Data Assessment*

The need for corrective action may be identified during either data validation or data assessment. Potential types of corrective action may include resampling by the field team or reanalysis of samples by the laboratory. These actions are dependent upon the ability to mobilize the field team and whether the data to be collected are necessary to meet the required QA objectives. If the data validator or data assessor identifies a corrective action situation that impacts the achievement of the project objectives, the AECOM Program Manager will be responsible for informing the appropriate personnel, including the MWRA Flounder Monitoring Project Area Manager.

C2. REPORTS TO MANAGEMENT

QA reports will be prepared by the AECOM Project QA Officer and submitted on an as-needed basis to the AECOM Program Manager. QA reports will document any problems identified during the sampling and analysis programs and the corrective measures taken in response. The QA reports will include:

- All results of field and laboratory audits
- Problems noted and actions taken during data validation and assessment
- Significant QA/QC problems, recommended corrective actions, and the outcome of corrective actions

A summary of QA issues, audit findings, and significant non-conformances will be included in quarterly status reports to the MWRA as part of the Corrective Action Log. Included in this report will be the dates when field, lab, and data technical system audits were conducted, and the dates on which any corrective actions resulting from these audits were completed.

D. DATA VALIDATION AND USABILITY

This section details the QA activities that will be performed to ensure that the collected data are scientifically defensible, properly documented, of known quality, and meet project objectives. Two steps are completed to ensure that project data quality needs are met:

- Data verification/validation
- Data usability assessment

D1. Data Review, Verification, and Validation

D1.1 Field Data

Verification of field data includes verification of sampling design, sample collection procedures, and sample handling. Field data will be reviewed by the Chief Scientist to ensure that the records are complete, accurate, and legible and to verify that the sampling procedures are in accordance with the protocols specified in the QAPP (refer to section D2.1of this QAPP for the specific elements reviewed).

D1.2 Laboratory Data

There are two primary sources of laboratory data for this project: (1) histology data read by Dr. Moore from the prepared tissue slides and (2) age analysis data provided by Mr. Jay Burnett at NOAA. Field observations on flounder, including weight, size, and external condition, together with laboratory data from (1) and (2) will be entered into the Access loading application by Dr. Moore.

After all data is entered, Dr. Moore will submit the loading application to AECOM for review. Ms. Stacy Doner will review the file and ensure that all required fields have been populated and will compare the electronic data with log sheets and other raw data supplied by Dr. Moore. This data file will then be forwarded to Mr. Greg Lescarbeau at Battelle for processing. A csv file that will be acceptable for loading by MWRA according to their rules will be generated by Battelle for AECOM. The csv file will be reviewed by Ms. Doner to ensure that all required fields have been properly converted from the Access application. Dr. Blake will then submit the file electronically to the MWRA Project Manager. Hard-copy of Dr. Moore's QA Report and other required information will be forwarded separately, together with any technical observations that Dr. Moore provides to accompany the Report.

D1.3 Data Management

The review process will include verification of manually entered data, quality control checks associated with loading applications, and script checks prior to exporting the data to MWRA. Detailed descriptions of these processes are included in Sections B10 and D2.

D2. VALIDATION AND VERIFICATION METHODS

D2.1 Field Data

Field records will be reviewed by the Chief Scientist to ensure that:

- Logbooks and standardized forms have been filled out completely and the recorded information accurately reflects the activities that were performed
- Records are legible and in accordance with good recordkeeping practices, i.e., entries are signed and dated, data are not obliterated, changes are initialed, dated, and explained
- Equipment calibration, sample collection, handling, preservation, storage, and shipping procedures were conducted in accordance with the protocols described in the QAPP, and that any deviations were documented and approved by the appropriate personnel

D2.2 Laboratory Data

As a part of data validation, each laboratory generating data for the flounder monitoring task will ensure that:

- The QC checks specified in Sections A7 and B5 were conducted and met the acceptance criteria
- All data that are hand-entered or typed will be 100% validated by qualified personnel prior to use in calculations or entry into the database
- All manual calculations will be performed by a second staff member to verify that calculations are accurate and appropriate
- Calculations performed by software will be independently verified at a frequency sufficient to ensure that the formulas are correct, appropriate, and consistent, and that calculations are accurately reported
- The supporting data is complete, accurate, and traceable

Once data have been generated and compiled, Dr. Moore will review the data to identify and make a professional judgment about any suspicious values. All suspect data will be reported, but flagged with a qualifier. These data may not be used in calculations or data summaries without the review and approval of Dr. Moore. No data measurements will be eliminated from the reported data or database and data gaps will never be filled with other existing data. The loss of any samples during shipment or analysis will be documented in the dataset package submitted to the MWRA and noted in the database.

D2.3 Data Management

Laboratory analytical data will be reviewed by AECOM prior to the electronic submission to MWRA. Data provided electronically to facilitate data handling will be verified against the hard copy data.

At Battelle, a second-level review of electronic submissions will be performed when the data 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.

For HOM7, a third-level review of the electronic data as generated by Battelle will be performed by the AECOM Laboratory Task Manager or Program Manager before results are submitted to MWRA. This review will serve to verify the completeness of the dataset submission and to ensure that project requirements are met for the analyses performed.

Finally, a fourth review by AECOM will take place after MWRA exports the data as a data report to verify that all data has been entered correctly in the EM&MS database.

D2.4 Project Deliverables

Upon completion of the verification/validation process, a dataset package will be prepared for submittal to MWRA. This package will include the following elements required for HOM7 flounder monitoring:

- Documentation of in-house checks (for example, listing any checking programs run)
- Cover letter describing any problems during loading
- Notes on all missing data and all data qualified as "suspect/invalid"
- List of problems encountered and corrective action taken
- Explanation of any outstanding issues resulting from the checks
- List of samples planned vs. collected, or measurements planned vs. reported
- QA Statement including a checklist of QA actions, and notes on deviations and corrective actions (electronic and signed hard copy)
- Summary statistics
- Table(s) of data submitted
- Exceptions report showing results of checks (for data sets submitted via the HOML application)

D3. RECONCILIATION WITH USER REQUIREMENTS

This element describes how the verified/validated project data will reconcile with the project DQOs, how data quality issues will be addressed, and how limitations on the use of the data will be reported and handled. The purpose of this section is to indicate the methods by which it will be ensured that the data collected for this investigation fall in line with the DQOs as described in Section A7 of this QAPP. To meet these DQOs, a combination of qualitative evaluations and statistical procedures will be used to check the quality of the data. These procedures will be used by the laboratory generating the data, by qualitative review by AECOM, and by statistical review by the Battelle Data Management Team.

The data generated must meet the MWRA's needs as defined in the project DQOs defined in Section A7 of this QAPP. The primary objectives for assessing the usability of the data are to ensure that (1) data denote conditions in Boston Harbor and Massachusetts and Cape Cod Bays, (2) all datasets are complete and defensible, and (3) data are of the quality needed to meet the overall objectives of the MWRA.

D3.1 Comparison to Measurement Criteria

D3.1.1 *Precision and Accuracy Assessment*

The accuracy and precision of the data generated during this program will be assessed by comparison to the data quality objectives specified in Section A7. Data that fail to meet the data quality criteria may necessitate sample reprocessing, analysis of archival material, sample recollection, or flagging of the data, depending on the magnitude of the nonconformance, logistical constraints, schedule, and cost.

D3.1.2 *Completeness Assessment*

Completeness is the ratio of the number of valid sample results to the total number of results planned for collection. The goal of this program is to generate valid, usable data. However, in environmental sampling and analysis, some data may be lost due to sampling location logistics, or field or laboratory errors. The overall completeness goal for the HOM7 Flounder Monitoring Program is 100% of planned samples to be collected and analyzed. The AECOM Laboratory Task Manager will assess the completeness of the overall data generation against the project goals. Following completion of the sampling, analysis, and data review, the percent completeness will be calculated and compared to the project objectives stated in Section A7.2 using the following equation:

$$\% \text{ Completeness} = \frac{\text{Number of valid/usable results obtained}}{\text{Number of valid/usable results planned}} \times 100$$

If this goal is not met, data gaps may exist that will require evaluation to determine the effect on the intended use of the data. Sample re-analysis, analysis of archived material, and/or re-collection of the sample may be appropriate depending on criticalness of the missing data, logistical constraints, cost, and schedule.

D3.1.3 *Representativeness*

Representativeness expresses the degree to which data accurately and precisely denotes a characteristic of a population, parameter variations at a sampling point, a process condition, or an environmental condition within a defined spatial and/or temporal boundary.

Representativeness of the field data will be assessed by verifying that the sampling program was implemented as proposed and that proper sampling techniques were used.

The assessment of representativeness in the laboratory will consist of verifying that the proper analytical procedures and appropriate methods were used.

D3.2 Overall Assessment of Environmental Data

Data assessment will involve an evaluation to determine if the data collected are of the appropriate quality, quantity, and representativeness for the purposes required by the MWRA. This evaluation will be performed by the AECOM Program Manager in concert with other users of the data. Data generated in association with QC results that meet these objectives will be considered usable. Data that do not meet the objectives and/or the data validation criteria might still be usable. This assessment may require various statistical procedures to establish outliers, correlations between data sets, adequate sampling location coverage, etc., in order to assess the effect of qualification or rejection of data. The effect of the qualification of data or loss of data deemed unacceptable for use, for whatever reason, will be discussed and decisions made on corrective action for potential data gaps.

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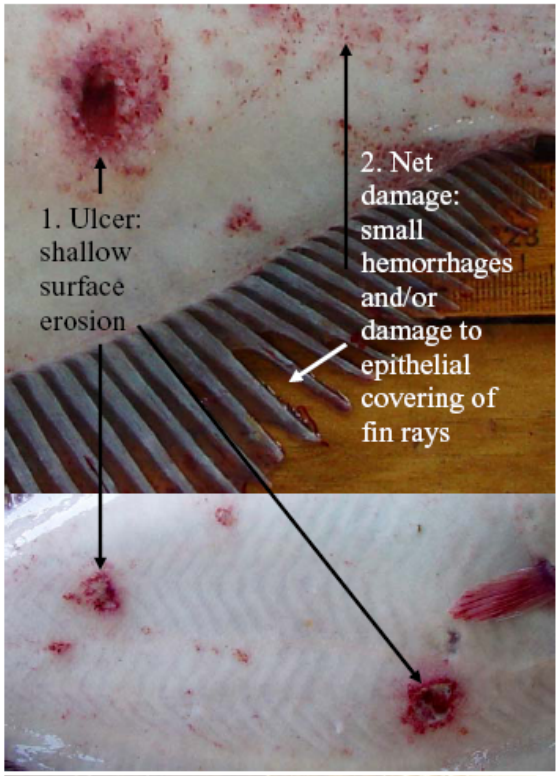
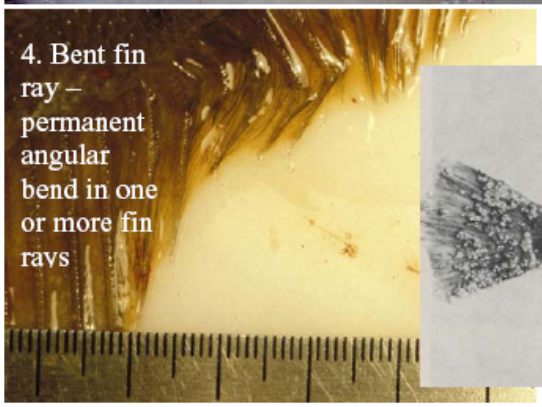
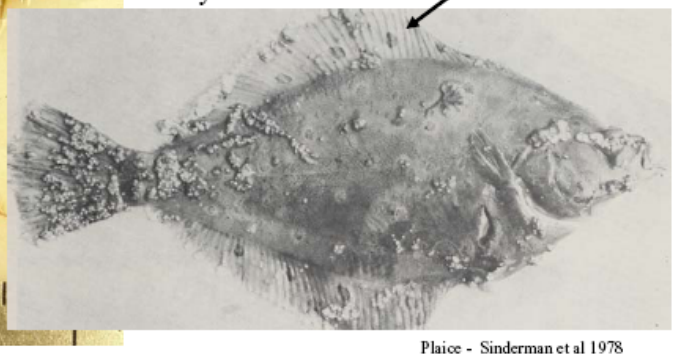
Appendix A
Guidance for Recording External Lesions in Flounder

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.

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