Quality Assurance Project Plan

for

Fish and Shellfish Monitoring: 2014-2016

Massachusetts Water Resources Authority
Environmental Quality Department
Report 2016-06

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QUALITY ASSURANCE PROJECT PLAN

for

Fish and Shellfish Monitoring: 2014-2016

MWRA Harbor and Outfall Monitoring Project
Contract No. OP216B

Prepared for

Massachusetts Water Resources Authority
Environmental Quality Department
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April 2014
Revised July 2016

Report No. 2016-06
PROJECT MANAGEMENT
REVISION 1

A. PROJECT MANAGEMENT

A1. TITLE AND APPROVALS

QUALITY ASSURANCE PROJECT PLAN
for
FISH AND SHELLFISH MONITORING: 2014-2016

MWRA Harbor and Outfall Monitoring Project
Contract No. OP216B

Prepared by
Normandeau Associates, Inc.
and
Woods Hole Oceanographic Institution
April 2014
Revised July 2016

Review and Approvals

Signature on file	7/18/2016
Ms. Ann Pembroke Normandeau Program Manager	Date
Signature on file	7/18/2016
Mr. Robert Hasevlat Normandeau Project QA Officer	Date
Signature on file	7/18/2016
Dr. Michael Moore WHOI Principal Investigator (Flounder)	Date
Signature on file	7/18/2016
Mr. Kenneth E. Keay MWRA Project Manager	Date
Signature on file	7/18/2016
Dr. Douglas Hersh MWRA EM & MS Manager	Date

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A3. DISTRIBUTION LIST

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

Name, Affiliation	Date Sent
Kenneth E. Keay, MWRA	
Lucner Charlestra, MWRA	
Douglas Hersh, MWRA	
Yong Lao, MWRA	
Michael Moore, WHOI	
Ann Pembroke, Normandeau	
Robert Hasevlat, Normandeau	
Eric Nestler, Normandeau	
Erik Fel'Dotto, Normandeau	
Paul Geoghegan, Normandeau	
Vivian English, Experimental Pathology Laboratories	
Kenneth Simon, EnviroSystems	

A4. PROJECT AND TASK ORGANIZATION

A4.1 QAPP Introduction

This Quality Assurance Project Plan (QAPP) presents the organization, objectives, functional activities, and specific quality assurance (QA) and quality control (QC) activities associated with the Fish and Shellfish Monitoring that will be conducted in support of the Massachusetts Water Resources Authority (MWRA) Harbor and Outfall Monitoring Program (HOM9 Contract OP216B). This document also describes the specific protocols that will be followed for sampling, sample handling and storage, chain of custody, laboratory and field analyses, data review and validation, document management, data management, and data usability assessment. This QAPP was prepared in accordance with EPA guidance documents as described in Section A9.4.1 and is also based on prior HOM QAPPs that guided previous monitoring activities (Maciolek et al. 2010, Lao et al. 2012, Maciolek et al. 2008, Pembroke et al. 2006, Nestler et al. 2011, Nestler et al. 2013). Separate survey plans developed for each survey will supplement this QAPP. The survey plans will provide the operational details required to conduct each survey, and will describe participating staff, schedule details, and specific equipment.

This QAPP has been revised to record a change in the protocol used to determine the age of winter flounder. During 2014 and 2015, flounder age was determined using otoliths, with age based on scales used as a back-up method. During 2016, flounder age will be determined using scales only.

A4.2 Project Organization

The Fish and Shellfish Monitoring tasks will be accomplished through the coordinated efforts of experienced personnel from Normandeau Associates, Inc. (Normandeau), Woods Hole Oceanographic Institution (WHOI), additional subcontractors, and the MWRA Environmental Quality and Laboratory Services departments. Key project participants are listed here:

MWRA

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

- Dr. Betsy Reilley, 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.
- Dr. Lucner Charlestra, MWRA Project Area Manager for Fish & Shellfish, including Winter Flounder Monitoring.
- Ms. Wendy Leo, MWRA Environmental Monitoring and Management System (EM&MS) Database Manager.
- Dr. Yong Lao, MWRA Department of Laboratory Services (DLS). Dr. Lao will be responsible for all tissue chemistry laboratory analyses.

Normandeau

- Ms. Ann Pembroke, Normandeau 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. She is responsible for ensuring that data collection and interpretation are scientifically defensible and for responding to technical challenges as they arise.

- Ms. Marcia Bowen, Normandeau 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.
- Mr. Robert Hasevlat, Normandeau Project Quality Assurance (QA) Officer, is responsible for reviewing the QAPP, survey reports, and data reports. He will also review QA Statements submitted by subcontractors for quality, completeness, and adherence to the QAPP. As Normandeau's Health & Safety Officer, Mr. Hasevlat will also review and approve the health and safety plans and procedures for the project.
- Mr. Eric Nestler, Normandeau's Assistant Program Manager, will prepare the QAPP, oversee data management, and assist Dr. Moore with data analysis and report preparation.
- Mr. Paul Geoghegan is Normandeau's Task Manager for the lobster survey and mussel bioaccumulation monitoring. He will also provide the overall technical review for the project.
- Mr. Erik Fel'Dotto, Normandeau's Field Manager for the mussel survey, will oversee the field aspects of the mussel bioaccumulation survey.
- Dr. Mark Mattson is Normandeau's Statistical Advisor for the project and will assist as needed with data analysis and interpretation.

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.

Additional Participants

- Mr. Mark Carroll, Captain of the F/V *Harvest Moon*, will provide navigational support for the field surveys.
- Ms. Vivian English, Experimental Pathology Laboratories (EPL), will oversee the preparation of histological slides under subcontract to WHOI.
- Mr. Kenneth Simon, EnviroSystems Inc. (ESI), will oversee the laboratory processing (e.g., tissue resection, collection of condition data, sample compositing) of flounders, lobsters, and mussels selected for chemical analyses during 2015.

Contact information and specific project roles for project participants are summarized in Table 1.

Table 1. Personnel Responsibilities and Contact Information.

Name/ Affiliation	Address	Project Area Assignment	Contact Information
MWRA			
Dr. Betsy Reilley	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 Betsy.Reilley[at]mwra.com
Mr. Kenneth Keay		Project Manager	Ph: (617) 788-4947 Fax: (617) 788-4888 Kenneth.Keay[at]mwra.com
Dr. Lucner Charlestra		Fish and Shellfish Project Area Manager	Ph: (617) 788-4943 Fax: (617) 788-4888 Lucner.Charlestra[at]mwra.com
Dr. Douglas Hersh		EM&MS Manager	Ph: (617) 788-4945 Fax: (617) 788-4888 Douglas.hersh[at]mwra.com
Dr. Yong Lao	Department of Laboratory Services MWRA Deer Island Treatment Plant 190 Tafts Avenue Winthrop, MA 02152	Client Services	Ph: (617) 660-7841 Fax: (617) 660-7960 Yong.Lao[at]mwra.com
Normandeau			
Ms. Ann Pembroke	Normandeau Associates, Inc. 25 Nashua Road Bedford, NH 03110	Program Manager **(All Tasks)**	Ph: (603) 637-1169 Fax: (603) 472-7052 apembroke[at]normandeau.com
Mr. Eric Nestler		Assistant Program Manager; QAPP Editor (**Task 3**), Data Manager (**Task 4**), Scale Analysis (**Task 11.1**), Data analyst **(Tasks 14 and 15)**	Ph: (603) 637-1146 Fax: (603) 472-7052 enestler[at]normandeau.com
Mr. Paul Geoghegan		Task Manager - Shellfish Monitoring (**Tasks 9 and 10**)	Ph: (603) 637-1163 Fax: (603) 472-7052 pgeoghegan[at]normandeau.com
Mr. Robert Hasevlat		Project QA Officer **(Tasks 3 and 4)**; Project Health and Safety Officer	Ph: (603) 637-1142 Fax: (603) 472-7052 rhasevlat[at]normandeau.com
Mr. Erik Fel'Dotto	Normandeau Associates, Inc. 43 Harbor Road Hampton, NH 03842	Field Manager - Mussel Survey; **(Task 10)**	Ph: (603) 926-7661 Fax: (603) 929-1434 efeldotto[at]normandeau.com
Dr. Mark Mattson	Normandeau Associates, Inc. 30 International Drive, Suite 6 Portsmouth, NH 03801	Project Biostatistician **(Tasks 14 and 15)**	Ph: (603) 319-5307 Fax: (603) 334-6397 mmattson[at]normandeau.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, 4, 8, 11, 14, and 15)**	Ph: (508) 289-3228 Ph: (508) 989-3575 (cell) Fax: 508 457 2089 mmoore[at]whoi.edu
Additional Subcontractors			
Mr. Kenneth Simon	EnviroSystems, Inc P.O. Box 778 One Lafayette Road Hampton, NH 03842	Tissue contaminant analysis preparation **(Task 12)**	Ph: (603) 926-3345 Fax: (603) 926-3521 ksimon[at]envirosystems.com
Mr. Mark Carroll	Not available at this time.	Captain, F/V *Harvest Moon* **(Task 8.2)**	Ph: (978) 985-7645
Ms. Vivian English	Experimental Pathology Laboratories, P.O. Box 474, Herndon, VA 20172	Histological preparation (**Task 11.2**)	Ph: (703) 471-7060 x222

A4.3 Task Organization

The following tasks covered by Contract OP216B are relevant to Fish and Shellfish Monitoring:

- Task 2 Project management, coordination, and tracking
- Task 3 QA Project Plan development
- Task 4 Data quality control and data set submission
- Task 8 Flounder survey
- Task 9 Lobster survey
- Task 10 Mussel bioaccumulation survey
- Task 11 Flounder histology analysis
- Task 12 Tissue contaminant analysis preparation
- Task 14 Annual technical meeting
- Task 15 Synthesis reports

Task 2 (management) is not further addressed in this QAPP. Task 3 (QAPP preparation) is described in section A9.4.1. Task 4 (data management) is discussed in section B10. Tasks 8, 9, 10, 11, and 12 (surveys and sample analyses) are discussed in detail in sections A6 and B2. Task 14 includes participation in an Annual Technical Workshop and is not further described. Task 15 (annual report) is described in section A9.4.6.

Lobster (Task 9) and mussel (Task 10) surveys, and the preparation of flounder, lobster, and mussel tissues (Task 12) for analysis of chemical contaminants will be part of this project in 2015 only. The chemical analyses will be performed by MWRA's Department of Laboratory Services (DLS), and MWRA will also prepare the report on the findings.

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. Such activities resulted in discernible impacts on harbor fauna. By the 1980's, winter flounder (*Pseudopleuronectes americanus*) collected in the harbor had a high prevalence of fin rot and lesions, and the incidence of liver tumors in this bottom-dwelling species was among the highest 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).

A5.2 Fish and Shellfish Monitoring

The Harbor and Outfall Monitoring (HOM) program is a requirement of MWRA's NPDES permit to discharge treated wastewater. Discharge from MWRA's ocean outfall has the potential to introduce various contaminants to the Massachusetts Bay ecosystem. Effects may be apparent as increased body burdens in marine organisms or as increased susceptibility to diseases as evidenced by lesions or tumors. Thus, fish and shellfish monitoring is a key component of the HOM. The purpose of fish and shellfish monitoring is to document conditions in the vicinity of the outfall and in farfield areas, to aid in the evaluation of outfall impact assessment. Health of key marine biota, as represented by winter flounder, American lobster (*Homarus americanus*), and blue mussel (*Mytilus edulis*), has ramifications both to the ecosystem and to human use and health.

Baseline monitoring of fish and shellfish began in 1991 for flounder and mussels, and in 1992 for lobster. After start-up of the offshore outfall in September 2000, the first year of "post-diversion" monitoring was in 2001. Surveys were conducted annually during the baseline period through 2003; currently, flounder surveys for health and condition (including histopathology) continue on an annual basis, while lobster surveys, mussel surveys and flounder tissue contaminant analyses are now done every three years (next surveys in 2015). This long-term biomonitoring program provides data that may be used to identify both spatial and temporal patterns, to assess potential impacts of MWRA's effluent discharge to fish and shellfish in Massachusetts Bay.

A5.3 Objectives and Scope

The overall objective of the fish and shellfish monitoring is to define the condition of fish and shellfish health in terms of the presence of disease (external and internal; winter flounder and lobster), and organic

and inorganic (metal) contaminant concentrations in the liver (winter flounder), hepatopancreas (lobster), and edible tissue (winter flounder, lobster, and mussel) of these selected organisms.

Key objectives of this monitoring effort include evaluation of the following:

- Spatial and temporal patterns of external and internal physical abnormalities and meristics in flounder
- Spatial and temporal patterns of external physical abnormalities and meristics in lobsters
- Spatial and temporal patterns of biological (flounder, lobster, and mussel) uptake of chemical constituents that may be linked to the outfall, and
- Relationship of body burdens to environmental and human health thresholds.

The MWRA Contingency Plan specifies how MWRA and the regulatory agencies will respond if monitoring results indicate a possible environmental problem (MWRA 2001). The Contingency Plan is an attachment to the Memorandum of Agreement among the National Marine Fisheries Service, EPA, and MWRA. Warning-level thresholds for specific monitoring parameters that are 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 in flounder, lobster and mussels (MWRA 2001).

Further background on the HOM Fish and Shellfish program, including program objectives, and results of previous monitoring activities, can be referenced in the following documents:

Hall M, Lao Y, Moore M, Carroll S. 2010. 2009 fish and shellfish report. Boston: Massachusetts Water Resources Authority. Report 2010-06. 71p.

Lao, Y, J Constantino, W Leo, MF Delaney, P Epelman, and S Rhode. 2012. Quality Assurance Project Plan (QAPP) for Chemistry Analyses for Fish and Shellfish Monitoring, Revision 1 (2012). Boston: Massachusetts Water Resources Authority. Report 2012-04. 46 pp.

Moore MJ, Nestler EC, and AE Pembroke. 2012. Flounder Monitoring Report: 2012 Results. Boston: Massachusetts Water Resources Authority. Report 2012-12. 17 pp.

Moore MJ, Nestler EC, and AE Pembroke. 2013. Flounder Monitoring Report: 2013 Results. Boston: Massachusetts Water Resources Authority. Report 2013-17. 17 pp.

Werme C, Hunt CD. 2008. Outfall monitoring overview BACKGROUND. Boston: Massachusetts Water Resources Authority. Report 2008-18. 54p.

A6. PROJECT/TASK DESCRIPTION

A6.1 Project Overview

To determine the body burden of toxic substances and to assess the physiological status of winter flounder (*Pseudopleuronectes americanus*) and lobster (*Homarus americanus*), specimens for analysis will be collected from Boston Harbor, Massachusetts Bay, and Cape Cod Bay (Figures 1 and 2, Table 2). The bioaccumulation of toxic substances in blue mussel (*Mytilus edulis*) will also be investigated. Arrays of mussels from reference locations will be deployed for 40 and 60 days in Boston Harbor and the Bays (Figure 3, Table 2).

During 2014 and 2016, only winter flounder will be collected, and chemical analyses will not be run. Flounder, lobster, and mussel surveys will all be conducted during 2015, including tissue preparation for contaminant analyses for each species. Tissue contaminant analysis will be performed by MWRA's Department of Laboratory Services (DLS).

The following five survey and analysis tasks will be performed:

1. Flounder Survey (2014, 2015 and 2016) – Task 8
2. Lobster Survey (2015) – Task 9
3. Mussel Bioaccumulation Survey (2015) – Task 10
4. Flounder Histology Analysis (2014, 2015 and 2016) – Task 11
5. Tissue Contaminant Analysis Preparation (2015) – Task 12

Normandeau will conduct Tasks 8-12 associated with the fish and shellfish monitoring following the protocols defined in the contract scope in order to maintain comparability with the historical database. Specific objectives for each of the five tasks included in this program are described below. Survey plans will be submitted to MWRA a minimum of two weeks prior to scheduled work to ensure that any questions that may arise about the plans can be addressed.

A6.2 Winter Flounder Survey (Task 8)

The flounder survey (Task 8.2) will be conducted on the FV *Harvest Moon*, operated by Captain Mark Carroll. Trawls will be made in late April and/or early May of 2014, 2015 and 2016 at four locations (Figure 1, Table 2): (1) Deer Island Flats, (2) off Nantasket Beach, (3) near the offshore outfall site, and (4) Eastern Cape Cod Bay. Fifty sexually mature (i.e., >300 mm total length) winter flounder will be collected at each station. The scientific crew will consist of Dr. Moore and a Normandeau fisheries technician to facilitate processing of the flounder.

Initial processing of the flounder (Tasks 8.3 through 8.5) will be conducted aboard the vessel. Upon collection, flounder will be properly maintained aboard the vessel, and those selected for processing will be killed by cervical section. Each flounder will be given a unique sample control number so that all data will be properly ascribed to a given flounder. Flounder will be measured and weighed, and the gender and external condition of each will be examined. Abnormalities such as fin rot, bent fin, or other external evidence of disease will be scored from 0 to 4, where: 0 = absent; 1 = minor; 2 = moderate; 3 = severe; and 4 = extreme. Otoliths (2014 and 2015) and scales (2014-2016) will be removed from each flounder for age determination in the laboratory.

Figure 1. Flounder monitoring locations for 2014, 2015, and 2016.

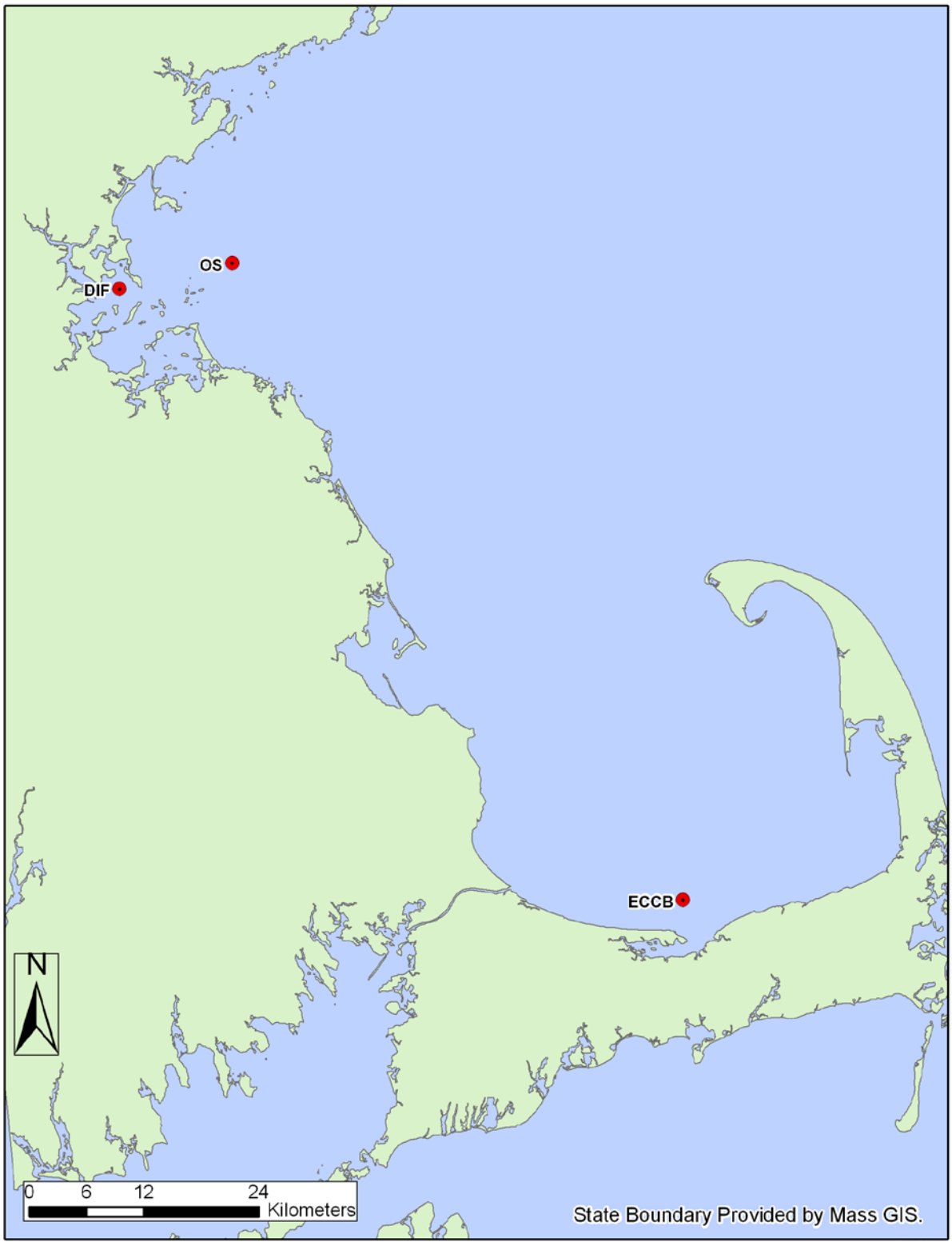


Figure 2. Lobster monitoring locations for 2015.

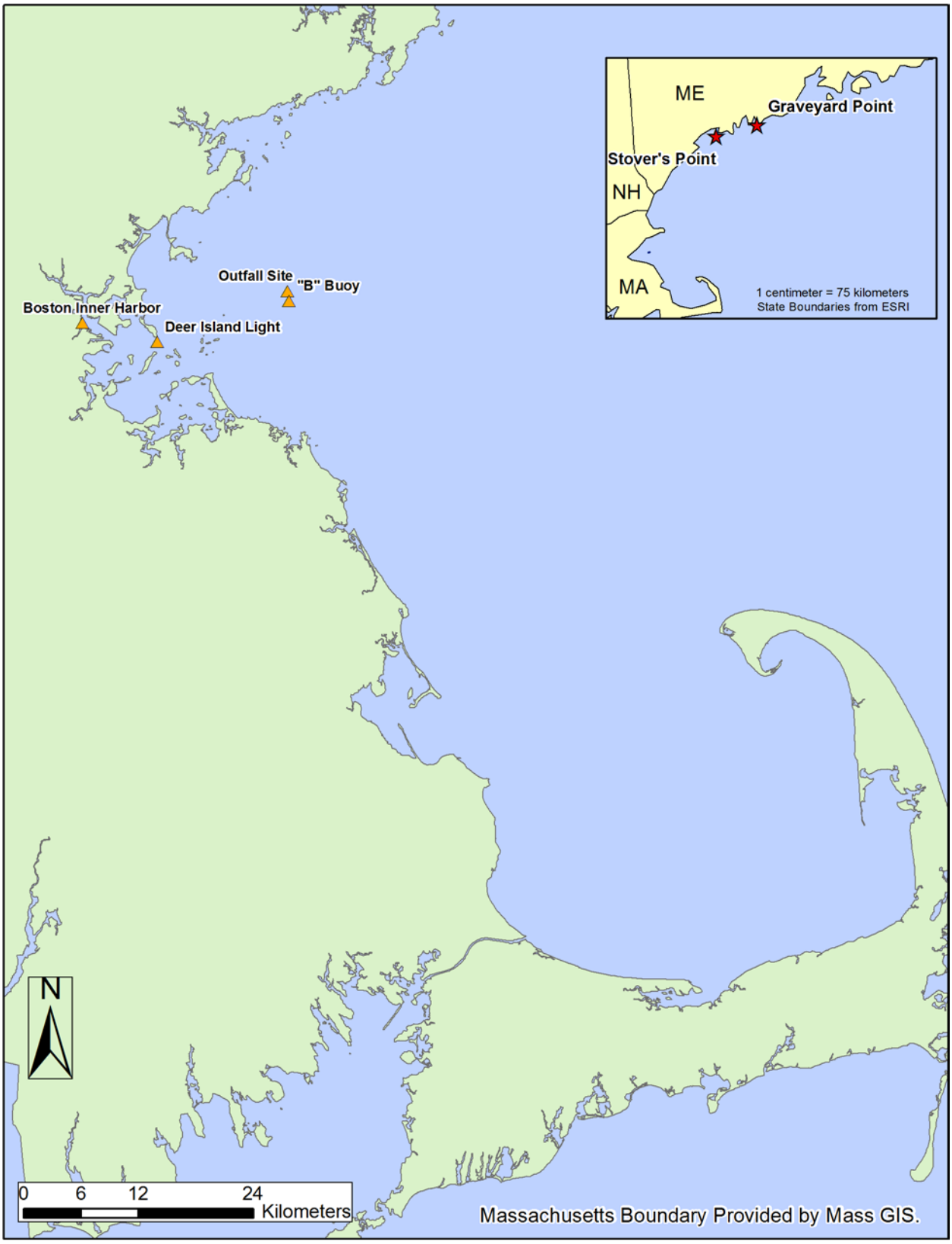


Figure 3. Mussel collection and deployment locations for 2015.

Table 2. Sampling and Locations for Flounder, Lobster, and Mussel Surveys.

Station ID	Station #	Sampling Site	Location		Survey Type		
			Latitude	Longitude	Flounder	Lobster	Mussel
DIF	1	Deer Island Flats (Boston Harbor)	42°20.4'	70°58.4'	*	*	
DIL	1M	Deer Island Light	42°20.4'	70°57.2'			*
NB	2	Off Nantasket Beach	42°17.6'	70°52.2'	*		
OS	4	Outfall Site	42°23.1'	70°49.3'	*	*	
OSM†	M4	Outfall Site (60 m at OS)	42°23.15'	70°47.92'			*
ECCB	5	East Cape Cod Bay	41°56.2'	70°06.6'	*	*	
IH	6	Boston Inner Harbor	42°21.5'	71°02.9'			*
SP	SP	Stover's Point, ME	43º45.1'	69º59.9'			R
PE	PE	Graveyard Point, Pemaquid, ME	43°52.8'	69°31.1'			R
LNB	B	"B" Buoy	42°22.67'	70°47.13'			*

* = Sampling Site for Survey. R = Reference Site for Collection of Mussels for Deployment during Bioaccumulation Survey. Exact location will be determined by availability of mussels.† Specific deployment locations will be selected from eight candidate stations (OS-M1, OS-M2,…OS-M8) by the MWRA Fish and Shellfish Project Area Manager when the survey plan is prepared. These stations are here referred to collectively as "OSM".

Flounder that are not selected for chemical analysis will have their livers examined, and liver samples will be collected and preserved for histopathological examination of the tissue.

In 2015, 15 flounders per site will be randomly selected for chemical analysis. Following initial shipboard processing and data collection (e.g., measurements, external conditions, scale removal), these 60 flounder will be transported on ice by the field technician to EnviroSystems Inc. (ESI) in Hampton, NH. Liver and edible tissue (skin-free fillet) will be aseptically removed by ESI (Task 8.6) and samples will be frozen for chemical analysis. Livers will be examined (e.g., color, gross anomalies) and a subsample will be collected and preserved for histopathological analysis.

The Catch Per Unit Effort (CPUE) (the number of fish caught per minute of bottom time) will be calculated at each sampling 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 Fish and Shellfish Project Area 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).

Field operations for the flounder survey will adhere to NOAA Fisheries (NMFS) regulations to reduce collisions between ships and the critically endangered North Atlantic right whale (50 CFR 224.103 (c), and http://www.nero.noaa.gov/Protected/mmp/viewing/regs/). During transit between stations, the captain and Dr. Moore will maintain a careful lookout for vessels and marine mammals. As required of all vessels by NMFS's rules, the vessel will maintain a minimum distance of 500 yards from right whales. Although reporting of sightings of healthy right whales is voluntary for all vessels, reasonable effort will be made to report sightings within 12 hours by calling 866-755-6622, which reaches the North Atlantic Right Whale Sighting Advisory System (http://www.nefsc.noaa.gov/psb/surveys/SAS.html). In the event of substantive aggregations of right whales in Cape Cod bay in late April, Dr. Moore will confer with Normandeau and MWRA and delay the Eastern Cape Cod Bay station sampling if need be.

A6.3 Lobster Survey (Task 9)

Lobster (*Homarus americanus*) will be collected for gross examination and chemical analyses of tissues to determine health and tissue burden of contaminants. Three sites will be sampled (Figure 2, Table 2): (1) Deer Island Flats (Boston Harbor), (2) Outfall Site (offshore effluent outfall), and (3) Eastern Cape Cod Bay. Specimens will be collected during surveys conducted in July 2015. Since lobsters migrate out of Boston Harbor into Massachusetts Bay in September, sufficient lobsters may not be available at the Outfall site during July. Consultations will be made with Massachusetts Division of Marine Fisheries prior to July 2015 to determine if lobsters are available at both the Harbor and Outfall sites. If sufficient lobsters cannot be collected during July, additional surveys will be conducted in deeper water near the Outfall site in an attempt to collect lobsters as close as possible to the July target date.

To conduct the survey (Task 9.2), Normandeau has identified commercial lobstermen to deploy traps at each of the three sampling sites (Table 2). The contracted Lobstermen will be provided with coordinates for deployment of the traps and guidelines for the maximum distance (section A7.1.1) from these coordinates that traps may be placed. Traps will be left in place for several days.

When it is time to retrieve the traps to collect lobsters for processing (Tasks 9.3 and 9.4), a fisheries technician from Normandeau will accompany the lobstermen. The fisheries technician will use a portable d-GPS unit to confirm the locations where the traps are retrieved. Lobsters will be kept alive during the collection. At each sampling site, 21 commercially harvestable (i.e., proper sized, non-berried individuals) will be collected, and each lobster will be given a unique sample control number.

The fisheries technician will process the lobsters for physical characteristics (carapace length, gender, and gross external abnormalities, such as shell erosion, parasites, and external tumors) and these will be recorded on the lobster sample collection log. Abnormalities will be graded on a scale of 0-4 where 0 indicates no incidence and 1-4 indicate quartile coverage of lobster by the abnormality (1=25% coverage, 2=50% coverage etc.).

Commercial lobster boats are unlikely to provide contamination-free conditions for processing the lobsters. Thus, the field technician will collect the initial data and then transport the lobsters (on ice) to EnviroSystems where tissue samples will be obtained in a clean room environment. Weight measurements (in grams) and black gill disease assessments will be conducted in the lab by EnviroSystems.

A6.4 Mussel Bioaccumulation Survey (Task 10)

A mussel bioaccumulation survey will be conducted between late June and late August 2015 in order to assess short-term accumulation of contaminants potentially dispersed via the outfall. Normandeau staff will be responsible for all field components of this task.

Test mussels will be collected from Stover's Point or Pemaquid in Maine (Task 10.2, see Table 2). Normandeau will update its special license from Maine Department of Marine Resources so that sufficient mussels for the pre-survey and post-survey analyses can be collected (Table 3). In addition to the minimum number of mussels required for analysis, 15% more at each location will be included to account for loss due to mortality during the deployment. Recent bioaccumulation surveys recorded survival rates of 83-97% (Nestler et al. 2007), so a 15% contingency should be ample to ensure there are sufficient mussels for chemical analysis. Mussels will be measured to ensure that the average length is approximately 6 cm.

Table 3. Minimum number of mussels required for each location.

Site	Number of Replicates	Minimum Number of Mussels
Deer Island Light (DIL)	4 x (2 retrievals + 1 backup array)	300 + 15%
Inner Harbor (IH)	4 x 2 retrievals	200 + 15%
Outfall (OSM)	8 x (2 retrievals + 1 backup array)	600 + 15%
"B" Buoy (LNB)	4 x 2 retrievals	200 + 15%
Stover's Point (SP)	4	100
Total	**44 + 12**	**1400 + 15%**

While water quality at Stover's Point and Pemaquid ME is affected by fewer discharges than Massachusetts Bay, many contaminants can be transported from great distances atmospherically. Knowledge of baseline levels of contaminants in mussels (Task 10.3) deployed for this survey is critical to assessing MWRA outfall effects. Normandeau will randomly select (i.e., choose without bias) 100 mussels (four replicates of 25 individuals), assign unique sample control numbers to the replicates, and transfer the samples to the laboratory for processing.

Normandeau will deploy sufficient mussels (Task 10.4) at each of the five survey locations (Table 2) to allow for collection of 25 mussels per replicate at 40-day and 60-day deployments for chemical analysis. The preferred deployment period is 60 days, but past surveys have occasionally encountered problems (vandalism or entanglement with fishing gear or vessels) that resulted in the loss of mussels. To minimize the possibility of missing data, several measures will be taken. First, the arrays will be constructed using heavy-duty materials. Second, one extra array will be deployed at each critical location (Deer Island and Outfall). Third, sufficient mussels will be retrieved for chemical analysis from each site after 40 days of exposure. These mussels will be frozen and held as contingencies until the Fish and Shellfish Monitoring Report for 2015 has been completed by MWRA. Thus, approximately 1600 mussels of the appropriate size will be collected to account for these redundancies.

Mussels will be retrieved from each location after 40 days (early August) to be held in contingency in the event that arrays are lost or mortality is unusually high between 40 and 60 days (Task 10.5). A sample control number will be assigned to each replicate and the mussels will be frozen. The condition of the cages (degree of fouling) and mussels (percent survival) will be recorded. If fouling is excessive such that it compromises the flow of water through the cages or weighs the array down sinking it in the water column, the field crew will retrieve the remaining arrays from that location and clean the fouling organisms from the cages prior to replacing them in the water.

In late August 2015, 60 days after deployment, mussels will be retrieved from the remaining arrays (Task 10.6). As with the 40-day retrieval, condition of the cages and mussels will be determined. Live mussels will be selected to make up the requisite number of replicate samples from each location. A sample control number will be assigned to each replicate and samples will be transferred to the laboratory (on ice) for processing.

A6.5 Flounder Histology Analysis (Task 11)

Flounder age will be determined (Task 11.1) by otolith analysis performed by Woods Hole NMFS Age and Growth Unit in 2014 and 2015 or scale analysis by Normandeau Associates in 2015 and 2016. An assessment of the health of flounder collected in Boston Harbor and Massachusetts Bay will be made through histological examination of liver tissue (Tasks 11.2 and 11.3).

Samples will be taken from the livers of all 50 flounder collected at each of the four survey sites sampled during 2014, 2015, and 2016. 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).

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. The majority of flounder thresholds pertain to levels of tissue contaminants, which will be measured only in 2015 as described below. Only the Caution level threshold value (44.94, based on baseline years 1991-2000) for incidence of liver disease (%) is relevant to this flounder histology task.

A6.6 Tissue contaminant analysis preparation (Task 12)

EnviroSystems, Inc. (ESI), a toxicity and analytical laboratory located in Hampton NH will composite the tissue samples collected during 2012 prior to delivery to MWRA's DLS. ESI maintains an ISO level 8 clean room, with conditions that are suitable for analysis of trace contaminants, and consistent with the requirements of the National Status and Trends program.

A6.6.1 *Flounder (Task 12.1)*

During 2015, 15 flounder from each of 4 sampling sites will be processed for chemistry analysis. Within a given station, each flounder (individually identified) will be randomly assigned to one of three groups for compositing (a total of five flounder per composite). The same individuals will be used for both liver and fillet composites. Flounders will be dissected within one day of collection using ceramic instruments. The liver will be removed using a pre-cleaned (i.e., rinsed with 10% HCl, Milli-pore-Q (18 megohm) water, acetone, DCM, and hexane) titanium knife and examined for gross abnormalities. Three serial slices will be removed and preserved in formalin to be sent to Dr. Moore for histological analysis. The remaining tissue will be used as part of the composite sample. The liver from each of the pre-selected five flounders per station will be chopped finely with the titanium knife and approximately equal wet weights of liver from each fish will be combined to form one composite sample. Each composite will be placed in a clean sample container, labeled, and frozen.

Fillets will be removed from the flounder and the skin will be removed from the fillets using a pre-cleaned titanium knife. Approximately equal masses of top and bottom tissue will be used from each flounder that is part of each composite and the total amount of tissue from each fish making up each composite will be approximately the same. Fillet tissue from the five fish will be homogenized, placed in a clean sample container, labeled with DLS sample identifiers, and frozen.

Once tissue samples are composited, they will be and transported to MWRA's DLS for chemistry analysis. The portion of the liver retained for histopathology will be preserved in buffered formalin and sent to Dr. Moore. Tissue remaining will be labeled with the tissue type and the individual sample identifier for that flounder, and retained separately by DLS for each individual should further analysis be required (see section **B3.4)**.

A6.6.2 *Lobster (Task 12.2)*

During 2015, 21 lobster from each of 3 sampling sites will be processed for chemistry analysis. Within a given station, each lobster (individually labeled) will be randomly assigned to one of three groups for compositing (a total of seven lobsters per composite). Separate composites will be made of hepatopancreas and meat (claw and tail). Dissections will be made using quartz, ceramic, glass, or polytetrafluorethylene (PTFE) materials. Approximately equal weights of tissue from each lobster will be included in a composite sample. Prior to combining the tissue, tissue from each lobster will be finely chopped or homogenized; once combined, the tissue will be re-homogenized and then frozen. The same lobsters making up a given composite of hepatopancreas tissue will also be used to make up the meat composite.

Excess tissue not used in the composites will be labeled with the tissue type and the individual sample identifier for that lobster, and retained separately by DLS for each individual should further analysis be required (see section **B3.4)**. Composite samples will be labeled with DLS sample identifiers and transported to MWRA's analytical laboratory.

A6.6.3 *Mussels (Task 12.3)*

During 2015, soft-body tissue composites will be prepared for the pre-deployment mussels, as well as the 60-day exposure mussels. Composites of the 40-day exposure mussels will only be made if survival is too low after 60 days of exposure to allow sufficient tissue for chemical analysis. Each composite will be made up of 25 mussels. There will be four composites for pre-deployment, Deer Island, "B" Buoy, and Inner Harbor and eight composites from the outfall site. Mussels will be randomly allocated to composites within each station. Mussels will be shucked and residual attached material and byssal threads removed. All soft tissue, including fluids, will be placed into a labeled (with DLS sample identifiers) clean glass jar, homogenized, and frozen. Frozen samples will be transported to MWRA's analytical laboratory.

A6.7 Flounder Report (Task 15)

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. Spatial and temporal trends in liver lesions will be examined.

A6.8 Schedule of Activities and Deliverables

Fish and shellfish monitoring activities under this contract will span the period from the date of project initiation (January 17, 2014) until completion of the final report in 2017. Activities include field sampling and laboratory analyses, with deliverables consisting of a QAPP, survey plans, survey summaries, survey reports, sample analyses data submissions, data report reviews, and annual reports. Schedules for these activities and deliverables for 2014-2016 are outlined in Table 4.

Table 4. Schedule of Fish and Shellfish Monitoring Deliverables.

Task	Deliverable	Due Date
Quality Assurance Project Plan (Task 3)	QAPP	Draft: March 2014 Final: April 2014
Flounder Survey (Task 8)	Survey Plan Survey Cruise Survey Summary E-mail Survey Report	March 2014, 2015, 2016 April 2014, 2015, 2016 2 days after survey May 2014, 2015, 2016
Lobster Survey (Task 9)	Survey Plan Survey Cruise Survey Summary E-mail Survey Report	June 2015 July 2015 2 days after survey September 2015
Mussel Bioaccumulation Survey (Task 10)	Survey Plan Mussel Deployment Mussel 40-day Collection Mussel 60-day Collection Survey Summary E-mail Survey Report	May 2015 June 2015 Early August 2015 Late August 2015 2 days after survey September 2015
Flounder Survey Data (Task 4)	Survey Data Tissue Composite Data	May 15, 2014, 2015, 2016 May 15, 2015
Lobster Survey Data (Task 4)	Survey Data Tissue Composite Data	Both due 15 days after survey completion in 2015
Mussel Bioaccumulation Survey Data (Task 4)	Survey Data Tissue Composite Data	September 15,2015 September 15, 2015
Flounder Histology Analysis (Tasks 4 and 11)	Histology Data	August 15, 2014, 2015, 2016 (preliminary report for liver disease incidence at Outfall Site is due 60 days after survey)
Flounder Histology Data Report Review (Task 11)	Letter Report of Review Comments	September 2014, 2015, 2016
Annual Technical Meeting (Task 14)	Presentation	March 2015, 2016, 2017

Annual Flounder Report (Task 15)	Draft Report Final Report	October 2014, 2015, 2016 November 2014, 2015, 2016

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 precision, accuracy, representativeness, comparability, and completeness must be met. These elements can be defined as:

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

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

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, lobster, and mussel monitoring stations to be visited during this program will be the same as those visited in previous years (Nestler et al. 2013).

Quality objectives are given below. Details of how these criteria will be met for each component of the 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. The shipboard navigation system on the fishing vessel *Harvest Moon* will be used for flounder surveys. Although the vessel's navigation equipment should be accurate and suitable for consistently fixing the vessel's position to within 15 m 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. For lobster surveys, the commercial vessel's navigation system will be used, and coordinates will be verified with a handheld GPS unit operated by the Normandeau field technician. Lobster sampling will be conducted within 300 m of the station location targets, but will ultimately be based on the availability of individual organisms. Normandeau's differential GPS (dGPS) navigation system will be used to acquire navigation data for mussel surveys. The deployment of mussels will be conducted within 15 m of the targets as determined by Normandeau's dGPS.

A7.1.2 *Flounder Survey*

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.

A7.1.3 *Lobster Survey*

The quality objectives for the collection of lobster are that (1) only specimens of commercially harvestable (i.e., proper sized, non-berried individuals) American lobster (*Homarus americanus*) are collected and (2) all required lobster (21 specimens) are collected at each station.

The quality objectives for the handling of lobster samples are that (1) all lobster are externally examined and all abnormalities carefully noted, (2) samples are handled properly to prevent deterioration of tissues, and (3) samples are labeled accurately. Procedures for sample handling are detailed in section B3 of this QAPP.

A7.1.4 *Mussel Bioaccumulation Survey*

The quality objectives for the collection and deployment of mussels are that (1) sufficient numbers (approximately 1600) properly identified specimens of blue mussel (*Mytilus edulis*), measuring approximately six centimeters, are collected from the reference site (2) sufficient numbers of live mussels are collected at each station after 40 and 60-day deployments.

The quality objectives for the handling of flounder samples are that (1) samples are handled properly to prevent deterioration of tissues, and (2) 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.

A7.2.2 *Flounder Age Analysis*

The data quality objectives for the aging analysis of flounder 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

A7.2.3 *Tissue Contaminant Analysis Preparation*

The data quality objectives for the tissue contaminant analysis preparation for flounder, lobster, and mussels are that (1) all samples are processed, (2) all laboratory methods as detailed in section B2 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 Normandeau Program Manager and the Normandeau QA Officer/Health and Safety 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 otoliths or 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 on otoliths as the primary method of aging as described in Appendix B. In case the otoliths do not provide definitive age determination, scales will be used to age the fish according to the methods of Fields (1988).

In 2015, ESI personnel responsible for tissue contaminant analysis preparation for flounder, lobster, and mussels will be trained in “clean laboratory” procedures.

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 or in the EnviroSystems Laboratory 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. Data management, including data custody after initial collection, is discussed in section B10 of this QAPP.

All data developed in the laboratory by Dr. Moore 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 D1 and D2 of this QAPP. Dr. Moore will provide a QA Report with his data deliverables.

Chain-of-custody forms (Figure 4) will be used to track flounder, lobster, and mussel samples as described in section B3.2. The original forms will be returned to Normandeau for the HOM9 project files once sample processing is complete.

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 for the flounder survey, will be responsible for ensuring that all events occurring during the survey are adequately documented in the Survey Log. All station data collected during the survey will be recorded on the Station Log forms (Figure 5). The start/end tow times and vessel position will be recorded by hand from the on-board GPS navigation system. Flounder measurements and condition data will be recorded on the Sample Log forms (Figure 6), which also serve as the Chain-of-Custody form for histology and scale samples. Age data will be entered later in the laboratory. At the end of the survey, Dr. Moore will provide copies of the completed survey records to Normandeau for the HOM9 project files and to Mr. Hall (MWRA) for review. A separate log book will be 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 will transmit any relevant field information to Normandeau for inclusion in the Survey Report.

The Normandeau Chief Scientist for the lobster and mussel bioaccumulation surveys will ensure that all required data are collected during the surveys, and that the data are adequately documented in the Survey Log forms (Figures 7 and 8).

MWRA Harbor and Outfall Monitoring Project

Chain of Custody Form

Project Name:						Parameters				Preservative			
Project Number:													
Originating Contact:													
Originator Location:													
Final Destination:													
Sampler(s):													
		Replicate		Collection						Frozen			
Sample No.*	Station		Type	Date	Time								Comments
	Total												

Relinquished by: (signature)	Recieved by: (signature)	Relinquished by: (signature)	Recieved by: (signature)	Page __of__	Method of Shipment:
Printed Name:	Printed Name:	Printed Name:	Printed Name:	Copy forwarded to:	
Date:	Date:	Date:	Date:		

*Sample number will be preprinted on Chain of Custody form.

Figure 4. Chain-of-custody form for flounder, lobster, and mussel tissue contaminant analysis samples.

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.)	# of wf >300 mm	Ghost traps per tow	Total fish/ station	Total time (min)/ station	Fish/min for entire station

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 for all dates to generate these parameters.

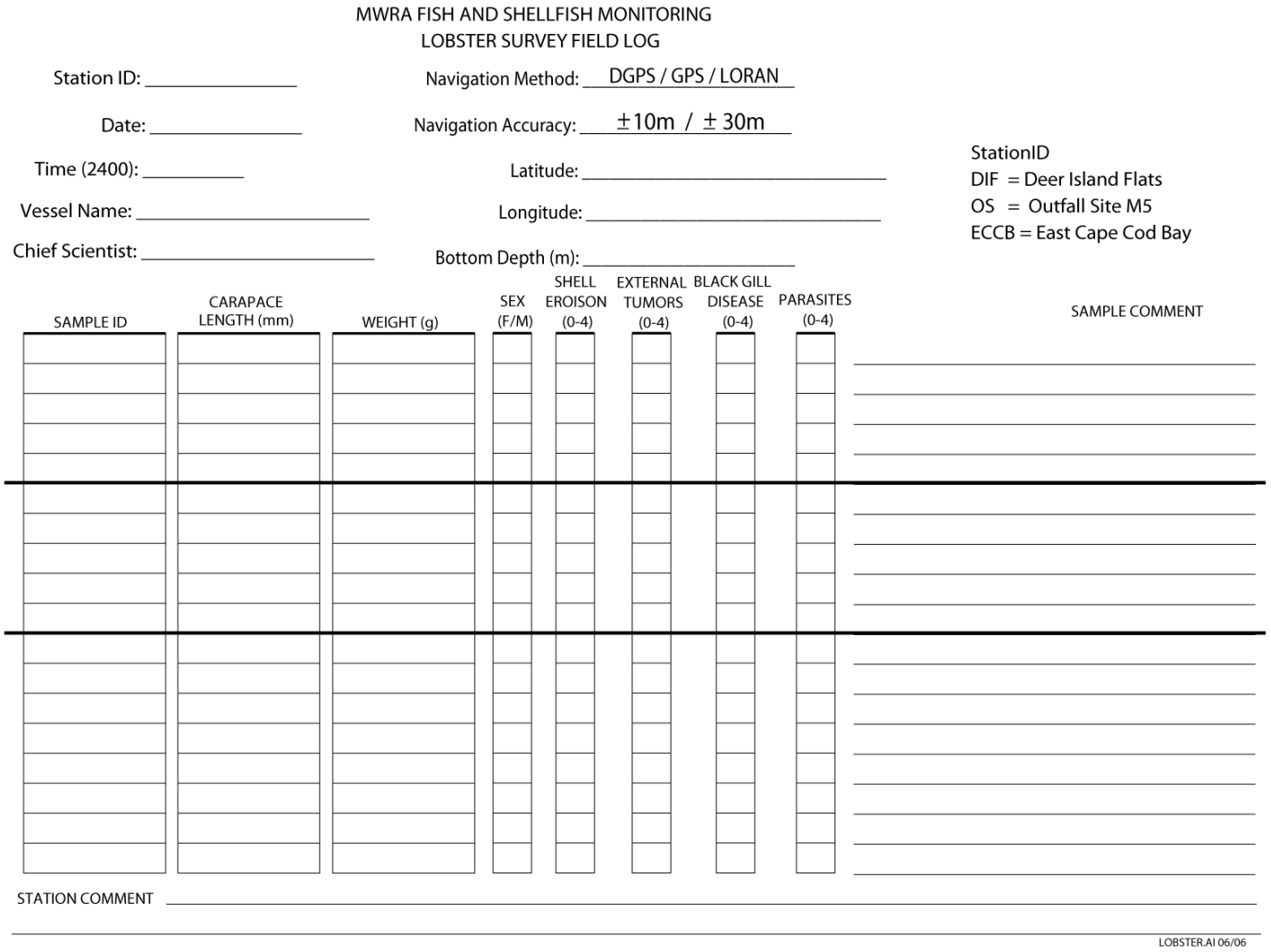
	Available Codes
VESSEL:	
NAVIGATION_CODE:	DGPS or GPS or LORAN
NAV_QUAL:	±10m and ±30m
VESSEL CREW:	
SCIENCE CREW:	

Figure 5. Field log sheet for flounder sampling (Station log sheet).

2011 External Lesions Data MWRA Harbor and Outfall Monitoring Program.

			Chain of Custody. Copy completed forms to go with each sample type	Scale_____ Histology_____ Chemistry_____		Relnq_____ ___/___/___ Rec'd _____ ___/___/___ ___/___/___ Rec'd _____ ___/___/___ Relnq_____ ___/___/___ Rec'd _____ ___/___/___								Relnq_____	
	ID	Station Name	Weight. (g)	TL mm	SL mm	Sex	Age	Fin_ Rot	Bent Fin	Ulcer (0-4)	Net (0-4)	Lympho. (0-4)	Liver Color*	Liver Gross+	Photos?
FF111	1001	Deer Island													
FF111	1002	Deer Island													
FF111	1003	Deer Island													
FF111	1004	Deer Island													
FF111	1005	Deer Island													
FF111	1006	Deer Island													
FF111	1007	Deer Island													
FF111	1008	Deer Island													
FF111	1009	Deer Island													
FF111	1010	Deer Island													
FF111	1011	Deer Island													
FF111	1012	Deer Island													
FF111	1013	Deer Island													
FF111	1014	Deer Island													
FF111	1015	Deer Island													

Figure 6. Sample log sheet for recording flounder data in the field.


MWRA FISH AND SHELLFISH MONITORING
LOBSTER SURVEY FIELD LOG

Station ID: _____ Navigation Method: DGPS / GPS / LORAN

Date: _____ Navigation Accuracy: ±10m / ± 30m

Time (2400): _____ Latitude: _____

Vessel Name: _____ Longitude: _____

Chief Scientist: _____ Bottom Depth (m): _____

StationID
DIF = Deer Island Flats
OS = Outfall Site M5
ECCB = East Cape Cod Bay

SAMPLE ID	CARAPACE LENGTH (mm)	WEIGHT (g)	SEX (F/M)	SHELL EROISON (0-4)	EXTERNAL TUMORS (0-4)	BLACK GILL DISEASE (0-4)	PARASITES (0-4)	SAMPLE COMMENT

STATION COMMENT _____

LOBSTER.AI 06/06

Figure 7. Lobster survey field log.

MWRA FISH AND SHELLFISH MONITORING
Mussel Survey Field Log Sheet

Date: _____ Deployment Date: _____

Station ID: _____ Exposure Time (days): _____

Chief Scientist: _____ Collection Start Time (2400): _____

Vessel Name: _____ Collection End Time (2400): _____

REP = 25 mussels per replicate

REP	CAGE FOULING %	CAGE SURVIVAL %
1		
2		
3		
4		
5		
6		
7		
8		

Deployment Depth (m): _____

Bottom Depth (m): _____

Navigation Method: DGPS / GPS / LORAN

Navigation Accuracy: ±10m / ± 30m

Latitude: _____

Longitude: _____

StationID
DIL = Deer Island
IH = Inner Harbor
M1 = Outfall Site M1
M2 = Outfall Site M2
M3 = Outfall Site M3
M5 = Outfall Site M5
LNB = "B" Buoy
SP = Stover's Point, ME

Fouling Codes:
0 = No fouling
1 = 1 - 10%
2 = 11 - 25%
3 = 26 - 50%
4 = 51 - 75%
5 = 76 - 100%

Survival Codes:
0 = None Alive
1 = 1 - 10%
2 = 11 - 25%
3 = 26 - 50%
4 = 51 - 75%
5 = 76 - 100%

Comments: _____

_____

_____

Mussel.ai 6/06

Figure 8. Mussel survey field log.

A9.3 Laboratory Records

There are four sources of laboratory records for this project: (1) in 2014 and 2015 age analysis data provided by NMFS are recorded by hand and provided to Dr. Moore, (2) in 2015 and 2016 age analysis data provided by Normandeau Associates are recorded by hand and then entered into Normandeau's data management system prior to delivery to MWRA; (3) histology data read by Dr. Moore from the slides and recorded electronically into Excel, and, (4) internal condition data collected by EnviroSystems for flounders (e.g. liver color) and lobsters (e.g. black gill disease) selected for tissue contaminant analyses during 2015.

Dr. Moore will enter the age (through 2015) and histology data (items 1 and 2 above) into an Excel file that has been prepared for these data, and used during prior surveys. Dr. Moore will ensure data quality through 100% verification of all hand-entered data.

The field sampling log sheets for flounders and lobsters that are selected for tissue contaminant analyses during 2015 will be delivered to the EnviroSystems laboratory along with the specimens. Data that could not be collected in the field (e.g. liver color for flounder, or black gill disease for lobster) will be recorded on the Survey Log forms in the laboratory.

A9.4 Reports and Data Submissions

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

- QAPP (Task 3)
- Survey plans (Tasks 8, 9, and 10)
- Survey summaries (Tasks 8, 9, and 10)
- Survey data submissions (Task 4)
- Survey reports (Tasks 8, 9, and 10)
- Sample analysis data submissions (Tasks 4 and 11)
- Review of MWRA generated data report (Task 11)
- Annual flounder report (Task 15)

A.9.4.1 *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 Normandeau 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.

A.9.4.2 *Survey Plan*

A survey plan will be prepared for each survey and will be submitted electronically 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

A.9.4.3 *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.

A.9.4.4 *Survey Report*

A survey report will be prepared and submitted within one month after each survey. The draft survey report will be submitted electronically to MWRA, and comments will be due two weeks after receipt of the draft report. 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 sampling (with starting and ending coordinates and time on station for flounder trawls)
- Sampling outcomes including a table with samples collected versus planned
- Summaries of individual measurements and gross conditions noted in the field
- Sufficiency of collected specimens 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 station locations

- 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.

A.9.4.5 *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 Normandeau and WHOI for review. Normandeau will submit a letter to MWRA with comments on the draft.

A.9.4.6 *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

A.9.5 Project Files

The project files will be the central repository for all documents relevant to sampling and analysis activities as described in this QAPP. Normandeau 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 Normandeau 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 Normandeau's servers, which are backed up on a daily basis.

Records associated with HOM9 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 fish and shellfish monitoring events is provided in Table 5.

B2. SAMPLING METHODS

B2.1 Flounder Collection

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 of 2014, 2015, and 2016. A commercial otter trawl will be towed by a commercial dragger (*e.g.*, F/V *Harvest Moon*, Captain Mark Carroll), 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 1).

Navigation data will be obtained from the on-board GPS 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 station log sheet (Figure 5). At all stations, the date and time will be recorded by hand into the field log sheet. The numbers of "ghost traps" collected per tow will also be recorded. These lost and abandoned traps (mostly old wire lobster traps) are commonly collected in trawls, which can reduce the efficiency of the survey.

Using a mechanical winch, the otter trawl will be brought on board and the contents unloaded onto the aft deck of the vessel. Specimens will be sorted taxonomically, but only winter flounder will be identified to species and retained: all other species will be returned to the sea, as will all winter flounder smaller than 300 mm. 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 NOAA Fisheries (NMFS) regulations to reduce collisions between ships and the critically endangered North Atlantic right whale (50 CFR 224.103 (c), and http://www.nero.noaa.gov/Protected/mmp/viewing/regs/). 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, Normandeau field technician, and Dr. Moore will maintain a careful lookout for vessels and marine mammals.

Table 5. Monitoring Parameters, Collection Frequency, Sample Containers, Preservation, and Holding Time Requirements

Organism	Parameter	Numbers of Sampling Units Total[a]/Sample[b]	Container	Shipboard or Laboratory Processing/Preservation	Holding Time from Collection
Winter flounder	Chemistry - liver - edible tissue	 15/3 15/3	Clean, labeled jar; Organics: Glass Inorganics: pre-cleaned polystyrene	Laboratory: Freeze, if not processed immediately	One year maintained frozen for all chemistry parameters (Thawed: all metals except Hg: 180-d; Hg: 28-d; SVOCs, PCBs, & Pesticides: 14-d to extraction & 40-d to analysis)
	Histology	50/50	Clean, labeled jar	Laboratory, Shipboard: 10% neutral buffered formalin	NA
	Age (otoliths/scales)	50/50	Age envelope	Shipboard: Clean mucous from sampling area of fish before taking scales	NA
	Visual	50/50	N/A	Shipboard: Describe qualitatively	NA
	- Biometrics weight standard length total length sex	50/50	N/A	Shipboard: Describe quantitatively	NA
Lobster	Chemistry - hepatopancreas - edible tissue	 21/3 21/3	Clean, labeled jar; Organics: Glass Inorganics: pre-cleaned polystyrene	Laboratory: Freeze, if not processed immediately	One year maintained frozen for all chemistry parameters (Thawed: all metals except Hg: 180-d; Hg: 28-d; SVOCs, PCBs, & Pesticides: 14-d to extraction & 40-d to analysis)
	Visual	21/21	N/A	Shipboard: Describe qualitatively	NA
	- Biometrics weight carapace length sex	21/21	N/A	Shipboard: Process immediately. Describe quantitatively	NA
Mussel	Chemistry - soft tissue	DIL: 100/4[c] OSM: 200/8 IH: 100/4 LNB: 100/4 SP: 100/4	Clean, labeled jar; Organics: Glass Inorganics: pre-cleaned polystyrene	Laboratory: Freeze if not processed immediately	One year maintained frozen for all chemistry parameters (Thawed: all metals except Hg: 180-d; Hg: 28-d; SVOCs, PCBs, & Pesticides: 14-d to extraction & 40-d to analysis)

a = total individual specimens collected per station.
b = total pooled (composite) samples to be analyzed per station.
c = number for each site to be collected 40 and 60 days after deployment, accept for those collected initially at reference site, SP.

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 9) will be measured and recorded on the field sample data sheet (Figure 6). 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.

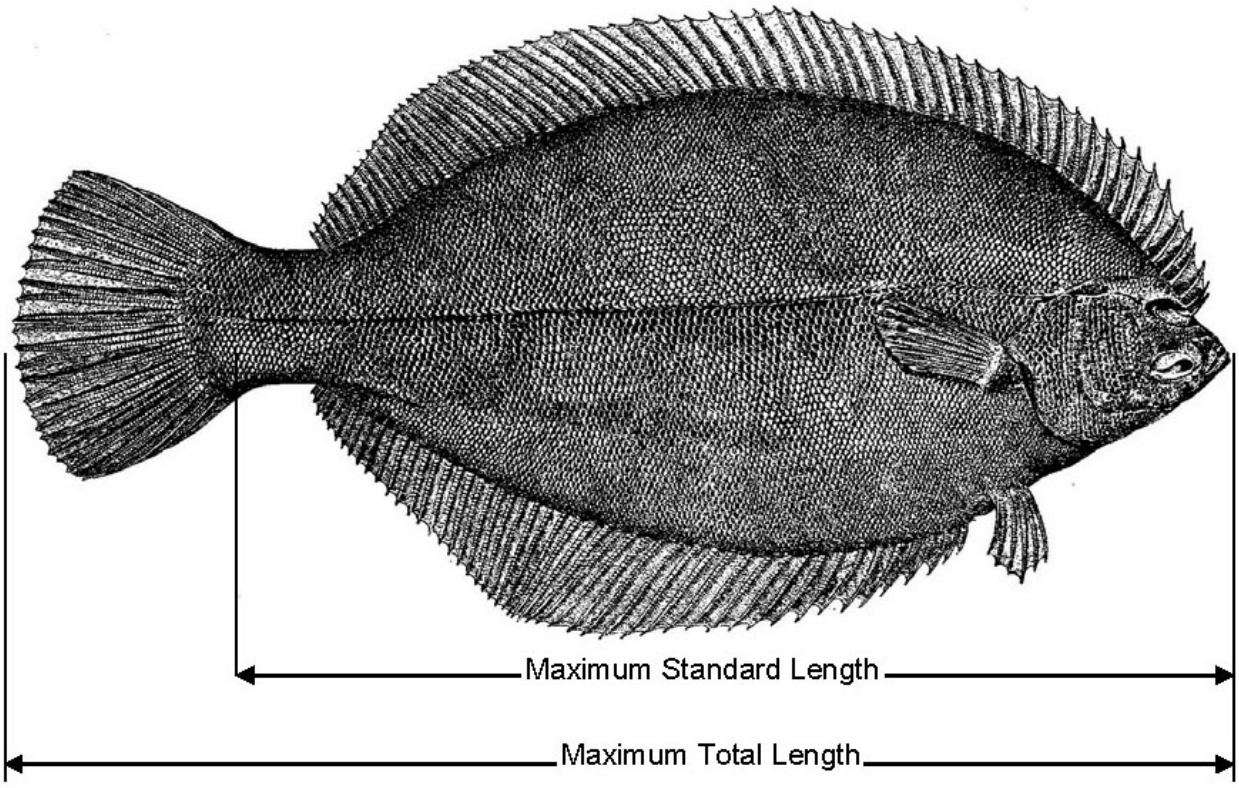


Figure 9. Length measurements for winter flounder.

For 2014 through 2015, otoliths (inner ear structures composed of calcium carbonate) will also be removed from each flounder following protocols described in Appendix B. For 2014 through 2015, otolith examination will be the primary methods for age determination and scales will be analyzed only if an age cannot be determined from the otolith. Otolith extractions will be performed after livers have been removed (see below), and otoliths will be placed in labeled sample envelopes. Scales will be also collected from each specimen for back up age determination through the analysis of growth rings (annuli) on the scales if the otoliths do not provide a definitive age. During 2016, flounder age will be determined using scales only. 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. Both otolith and scale samples will be subsequently sent to NMFS Age and Growth Laboratory in Woods Hole, MA for age determination (2014 and 2015). After receipt of the age data, the results will be entered on the data sheets, initialed, and dated. These results will be entered into the Excel file together with other results on the same data sheet. In 2016, aging will be conducted by Normandeau's Biological Laboratory, in Bedford, NH, using scales.

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 6). Three slices of liver from each flounder will be placed in a separate, clearly labeled histology cassette. Cassettes will be snapped shut and then rubber banded to ensure they remain closed. 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 6).

All 50 fish collected from each station in 2014, 2015, and 2016 will be processed for histopathology. In 2015 only, after the initial external examination and removal of scales, 15 fish from each station will be randomly selected for joint histological and chemical analysis. A random number generator will select 15 of the unique identifier numbers that are assigned to each fish. These 15 fish will be kept on ice and hand-delivered at the end of the day to EnviroSystems to be processed in the laboratory under clean, contaminant-free conditions. Normandeau will arrange with EnviroSystems for an after-hours delivery if necessary; or a courier from EnviroSystems will pick up the fish from the boat.

B2.3 Lobster Collection

Three sites will be sampled to collect lobster for chemical analyses during 2015: (1) Deer Island Flats (Boston Harbor), (2) Outfall Site (offshore effluent outfall), and (3) East Cape Cod Bay. Table 2 provides the sampling sites and locations. Figure 2 illustrates the sampling locations in Boston Harbor and Offshore.

Twenty-one lobsters will be collected per site. Historically it has not been possible to obtain the required lobsters at all the sites during July because of the seasonal onshore-offshore movements of this species. Therefore, several sampling events may be necessary at the outfall to obtain sufficient lobsters for analysis. Decisions about when to resample will be based on the survey reports provided under the water column monitoring program that include observations of lobstering activities and consultation with Massachusetts Division of Marine Fisheries. The following lobster collection procedures will be followed.

1. Commercial lobstermen will procure the lobsters for chemical analysis. The lobstermen will be provided with coordinates for deployment of the traps and guidelines for the maximum distance from these coordinates that traps may be placed (section A7.1.1). Traps will be left in place for several days.

2. When it is time to retrieve the traps, a fisheries technician from Normandeau will accompany the lobstermen. The fisheries technician will use a portable d-GPS unit to confirm the locations where the traps are retrieved and document the location on the lobster sample collection log (Figure 7).

3. Upon retrieval, lobster pots will be brought alongside the vessel to the gunnel (side railing). Specimens will be removed and measured on the gunnel (Figure 10). No specimens will be brought on board the vessel until it is determined that they have a carapace length of at least 3.25 in (legal length). In addition, any specimens that are of legal length but also contain eggs (berried) will be returned to the environment immediately.

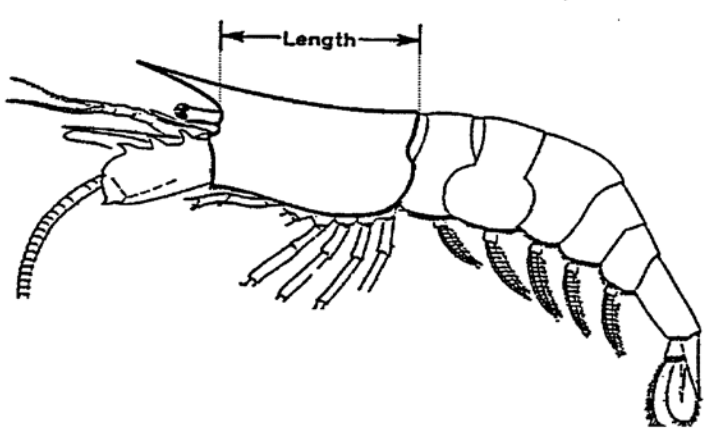


Figure 10. Carapace measurement of lobster

4. The fisheries technician will process the lobsters for physical characteristics (carapace length, weight, gender, and gross external abnormalities, such as shell erosion, parasites, and external tumors; to be recorded on the lobster sample collection log. These measurements and inspections will be made immediately upon capture to improve chances of survival.

5. Twenty-one specimens retained for processing will be banded with one band per claw. Each lobster will be given a unique sample control number to indicate event, year, survey, and site of collection.

6. During the survey, lobster specimens will be stored away from commercial lobsters in a separate container with site water.

7. Whole lobsters will be kept on ice and hand-delivered at the end of the day to EnviroSystems to be processed in the laboratory under clean, contaminant-free conditions. Normandeau will arrange with EnviroSystems for an after-hours delivery if necessary; or a courier from EnviroSystems will pick up the samples from the boat.

8. Weight measurements (in grams) and black gill disease assessments will be conducted in the lab by EnviroSystems. Lobsters with black gill disease show brown to black spots on the gills. In acute cases

gills may become completely brown or black with atrophy and necrosis. Historically, there has been no evidence of black gill disease and no project-specific protocols for grading this disease exist. Therefore, if any lobsters exhibit signs of black gill disease, the condition of the gill will be described qualitatively and photographed.

B2.4 Mussel Deployment and Collection

During late June of 2015, live blue mussels (*Mytilus edulis*) approximately 6 cm in length will be collected from the baseline (pre-survey) reference site in Stover's Point or Pemaquid, ME. Normandeau will update its license from the Maine Department of Marine Resources for the procurement of these mussels. One hundred mussels will be randomly selected for chemical contaminant analysis. The remaining mussels will be deployed and retrieved at four sites:

Four locations will be used for mussel deployment for subsequent chemical analyses: (1) Deer Island Light (~2 m above bottom), (2) Outfall Site – approximately 60 ±15 m from the offshore outfall (depth of ~10-15 m above bottom, water depth ~30 m (MLW)), (3) Boston Inner Harbor (1.5 – 4.5 m above bottom – Rise and fall with tide, so that it is at a constant depth below the water surface), (4) B Buoy (Boston Approach Buoy, 1 km south of offshore outfall; 10-15 m above bottom). Table 2 provides the sampling sites and locations. Figure 3 illustrates the sampling locations in Boston Harbor and Offshore.

Table 3 provides the minimum number of mussels required for each location. In addition to the minimum number of mussels required for analysis, 15% more at each location will be deployed to account for loss due to mortality during the deployment. Recent bioaccumulation surveys recorded survival rates of 83-97% (Nestler et al. 2007), so a 15% contingency should be ample to ensure that there are sufficient mussels for chemical analysis. Mussels collected for the bioaccumulation survey will measure approximately 6 cm (55-65mm) (Figure 11).

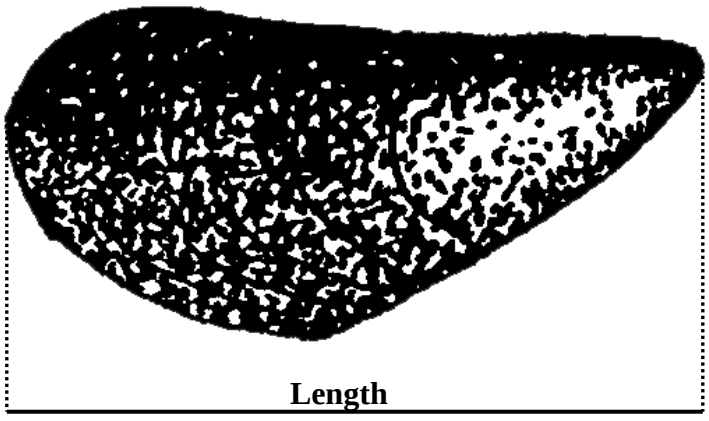


Figure 11. Length Measurement of Mussels.

Stover's Point, in Harpswell ME, was chosen as a reference site since water quality in this region is affected by fewer discharges than in Massachusetts Bay. Nonetheless, many contaminants can be transported from great distances atmospherically, and knowledge of baseline levels of contaminants in mussels deployed for this survey is critical to assessing MWRA outfall effects. Normandeau will

randomly select (i.e., choose without bias) 100 mussels (four replicates of 25 individuals) from the reference site, assign unique sample control numbers to the replicates, and pack the samples in ice prior to transport to the laboratory for chemical analysis.

Mussel populations have been declining in the Gulf of Maine, and insufficient numbers of mussels were found at Stover's Point in 2012. Furthermore, widespread PSP closures limited the collection of mussels from other locations. Mussels were eventually collected from Graveyard Point in Pemaquid ME. To help prevent this from reoccurring, regular and early communication with Maine Department of Marine Resources will be made to determine the status of PSP closures. In addition, reconnaissance visits will be made to Stover's Point and Graveyard Point in March during low tide to determine if mussel populations are sufficient for collection and consultation will also occur with local shellfish wardens. The results of these planning activities, including recommendations on where to collect mussels, will be presented in a summary report to MWRA via conference call.

Sufficient mussels at each of the locations identified in Table 2 will be deployed to allow for collection of 25 mussels per replicate at 40-day and 60-day deployments for chemical analysis. Table 3 provides minimum numbers of mussels required for each location. The preferred deployment period is 60 days but past surveys have occasionally encountered problems (vandalism or entanglement with fishing gear or vessels) that resulted in the loss of mussels. The following steps will be taken to minimize the possibility of missing data. First, mussel arrays will be constructed using heavy-duty materials. Second, one extra array will be deployed at each critical location (Deer Island and Outfall). Third, sufficient mussels for chemical analysis will be retrieved from each site after 40 days of exposure and transported to the analytical laboratory on ice. These mussels will be shucked and frozen to be held as contingencies until the Fish and Shellfish Monitoring Report for 2015 has been completed by MWRA. Thus, approximately 1600 mussels of the appropriate size will be collected during this survey. These redundancies in mussel collection should maximize the generation of usable data and minimize potential data gaps due to mussel losses.

Each array will be deployed on a separate mooring with enough mussels to provide sufficient tissue to complete the study. The locations of the arrays will be recorded using dGPS. Normandeau will retrieve mussels from each location after 40 days (early August) to be held in contingency in the event that arrays are lost or mortality is unusually high between 40 and 60 days. A sample control number will be assigned to each replicate and the mussels will be transported to the analytical laboratory on ice to be archived (frozen) until it is determined whether processing is required. The condition of the cages (degree of fouling) and mussels (percent survival) will be recorded. If fouling is excessive such that it compromises the flow of water through the cages or weighs the array down sinking it in the water column, the field crew will retrieve the remaining arrays from that location and clean the fouling organisms from the cages prior to replacing them in the water.

In late August 2015, 60 days after deployment, Normandeau will retrieve mussels from the remaining arrays. As in the 40-day retrieval, condition of the cages and mussels will be determined. Live mussels will be selected to make up the requisite number of replicate samples from each location. A sample control number will be assigned to each replicate and samples will be transported on ice to the EnviroSystems laboratory. Normandeau will arrange with EnviroSystems for an after-hours delivery if necessary; or a courier from EnviroSystems will pick up the samples from the boat.

B2.5 Flounder Processing for Tissue Contaminant Analysis

For stations where chemistry analyses are to be conducted, fifteen of the fifty fish will be randomly selected for joint histological and chemical analysis. These fish will be examined on board the collection vessel for external condition, including length (SL and TL), weight, and scales removed for aging. Because contaminant-free conditions cannot be found on board the vessel used for flounder collection, these fish will be placed alive on ice and transported to EnviroSystems, Hampton, NH, for on-shore processing for histological and chemical analysis. Fifteen unique sample identification numbers will be assigned to these fish during shipboard processing.

The flounder tissues will be removed at EnviroSystems under contaminant-free conditions (ISO level 8 clean room) (Appendix B). Processing for histology and internal conditions are described in section B2.2. A small portion of the liver will be removed (using a titanium or ceramic knife) and preserved for histology, such that most of the liver tissue can be maintained for chemical analysis. In addition, for chemical analysis, fillets (muscle) will be removed from the flounder, and the skin will be removed from the fillet using a pre-cleaned (i.e., rinsed with 10% HCL, Milli-Q (18 megohm) water, acetone, DCM, and hexane) stainless steel knife. Liver and fillet samples for chemical analysis will be maintained and identified as individual fish until being composited and homogenized.

Fillet composites will be made from equal aliquots (±10% by wet-weight) of the homogenate of 5 individual fish fillets using approximately equal masses of top and bottom tissue. The liver composite samples will contain approximately equal masses (5 grams) from each of the livers and will correspond to the composites made for the fillets. For fish with extremely small livers (< 5g wet weight), all available liver tissue will be used from such fish. Upon compositing, a new sample ID number will be generated to track the composite. There will be a total of three composites of each tissue for each station (Table 5). When the composites are prepared for analysis, sufficient tissue will be archived in the event that an anomalous result indicates the need for reanalysis. All fish tissue will be maintained frozen at EnviroSystems prior to transport to MWRA's DLS. Archived tissue will also be maintained frozen and transported to DLS.

B2.6 Lobster Processing for Tissue Contaminant Analysis

Whole lobsters will be delivered on ice to EnviroSystems where the hepatopancreas and meat (claws and tail) will be removed. Laboratory measurements and processing for internal conditions are described in section B2.3. From the 21 lobsters collected for each site, three composites of seven lobsters each will be made for each site. Two tissue types are to be analyzed per site (claw and tail meat, hepatopancreas), resulting in 18 composite samples (3 composites x 3 sites x 2 tissue types). The hepatopancreas will be removed using titanium, ceramic, or Teflon implements and frozen for chemical analysis. The tail and claw meat (edible tissue) will be stored frozen in the shells until processed in the laboratory. Homogenized samples of hepatopancreas or edible meat from each lobster in a pool will be quantitatively combined (±10% by wet- weight) to provide two composite samples per pool, one each of hepatopancreas and edible meat. Upon compositing, a new sample ID number will be generated to track the composite. Each composite will be placed in a sample container clearly identified with the unique sample identifier and maintained frozen prior to transport to MWRA's DLS. Material not required for analysis will be archived frozen and transported to DLS.

B2.7 Mussel Processing for Tissue Contaminant Analysis

EnviroSystems will shuck the mussels and homogenize the tissue to generate composite samples for each location. Each 25-mussel composite sample will be treated as an individual replicate. Composites for chemical analysis will be created as 4-8 composites (see Table 5) of 25 mussels each at each of the four site locations plus the baseline location.

Each individual mussel will be cleaned of attached material, all byssal threads removed, and all soft tissue including fluids placed directly into an appropriate container (500-ml I-Chem Certified clean bottle). Mussel composite samples will be prepared for chemical analyses by homogenization using titanium, ceramic, or Teflon coated equipment that has been rinsed with methanol and deionized water prior to use. Sample homogenates will then be split into appropriate containers for metals and organic analyses. Each composite will be placed in a sample container clearly identified with the unique sample identifier and maintained frozen prior to transport to MWRA's DLS. The portion of the composites not required for analysis will be frozen and archived at DLS in the event that results indicate the need to reanalyze any samples.

B3. SAMPLE HANDLING AND CUSTODY

B3.1 Sample Storage

Storage requirements for flounder, lobster, and mussel samples are described in Table 5 above.

B3.2 Sample Identification

Samples collected in the field will each be assigned a unique Sample ID. The Sample ID will identify the sample collected (i.e., a single flounder, a single lobster, or a mussel composite). The Sample ID will consist of the Event ID, the Station # (see Table 2), and a sequential number (001-050 for individual flounder, 001-021 for individual lobster, and 01-08 for mussel composites), concatenated. The five character Event ID will be unique to each survey, such as "FF151", with "FF" indicating that it is a flounder survey ("FL" for lobster survey and "FM" for mussel survey), "15" indicating the survey year, and "1" signifying the first survey of the year. Unique Bottle IDs will be assigned to identify sub-samples such as body tissue types for fish or lobster chemistry data, or liver slices for the flounder histology data.

B3.3 Sample Custody

During field collection, custody forms will be completed. Manual entries will be recorded in indelible ink in the data section of the Chain-of-Custody (COC) form. Based on the types of samples that will be collected, chain-of-custody forms will be generated for each sample type to track samples from collection to laboratory. An example COC form is provided in Figure 4.

The samples will remain in the custody of the Chief Scientist (designated for each survey) while in the field. Custody forms will accompany the samples when transferred from the field to the laboratory. All samples will be distributed to the appropriate laboratory personnel by hand or by Federal Express. When samples arrive at each of the laboratories, custody will be relinquished to the Laboratory Custodian. Upon receipt of the samples, the laboratory Sample Custodian will examine the samples, verify that sample-specific information recorded on the custody forms is accurate and that the sample integrity is uncompromised, log the samples into the laboratory tracking system, complete the custody forms, and sign the custody form so that transfer of custody of the samples is complete. Completed custody forms must be faxed to the Normandeau Project Manager within 24 hours of sample receipt. Any discrepancies between sample labels and transmittal forms, and unusual events or deviations from the QAPP will be documented in detail on the custody form and the Normandeau Project Manager and Laboratory Project Manager notified within 24 hours.

The laboratory custodian of samples for histologic analyses will be Dr. Michael Moore, of WHOI. Following each flounder survey, Dr. Moore 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 (during 2014 and 2015). During 2016, scales will be hand delivered under COC to Normandeau's Biological Laboratory, in Bedford, NH.

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. The signed original custody forms for histological samples and scales will be retained in Dr. Moore's project files.

EnviroSystems will maintain custody of flounder, lobster and mussel samples for tissue contaminant analysis preparation. The EnviroSystems laboratory custodian of samples will be responsible for receiving samples (by signing the COC) for tissue contaminant analysis preparation. The procedures described above in this section apply for sample receipt and log-in. When samples are composited, a new sample identification number from the label provided by DLS will be assigned to the composite sample and the DLS label applied to the composite sample container. DLS will provide Normandeau with an electronic list of the subsample unique identifiers (BOTTLE_ID) associated with the pre-printed labels. Using this listing of subsample IDs, samples (e.g., individual fish or lobsters; SAMPLE_ID) will be pre-assigned to composite samples (SAMPLEC_ID) and subsamples (BOTTLE_ID).Laboratory staff will prepare composite samples by following a printed table that associates samples to composite samples and subsamples. This will ensure an accurate accounting of which SAMPLE_IDs correspond to which DLS subsample identifiers. A complete description of sample identification for tissue chemistry composites is provided in Appendix C. The original COC forms will be submitted to DLS with the samples for tissue contaminant analysis. Following DLS's acceptance of these samples, copies of these COCs will be submitted to the Normandeau Project Manager and maintained in the MWRA project files, while the original COCs will be held by DLS.

B3.4 Sample Archival Policy

The types of materials that may be archived under this contract include flounder, lobster, and mussel tissues, and slides containing flounder liver sections and flounder scales used in aging. The archived tissues will be maintained frozen at DLS until the Fish and Shellfish Report for 2015 has been completed by MWRA. 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

Scale analysis, using methods described by Fields (1988) had been the preferred method historically, but recently, reading growth rings (annuli) on otoliths became the preferred aging method for older fish. In 2014 and 2015, the Age and Growth Unit of the NMFS conducted age determination using ototliths, following procedures described by Fields (1997), with missing data filled in using scales. In 2016, aging will be conducted using scales because of NMFS' limited availability. Scales will be pressed between two microscope slides, examined with a 46X power microfiche so that the technician can count annular rings.

B5. QUALITY CONTROL

B5.1 Sampling

B5.1.1 *Navigation*

Precision and Accuracy
All dGPS units have a design positional accuracy of 15 m. Based on manufacturer specifications and project experience, precision and accuracy objectives for navigation and station depth are presented in Table 6.

Table 6. Precision and Accuracy of Navigation Data.

Sensor	Units	Range	Accuracy	Precision
Fathometer (depth)	m	0 to 200	2	0.1
dGPS Navigation	degree	Coastal	$9x10^{-5}$ deg (10 m)	$1.8x10^{-5}$ deg (2 m)

Representativeness
The representativeness of the sampling program design is detailed in the Outfall Monitoring Plan (MWRA 1997) and defined by the results collected since 1992.

Comparability
Latitude/longitude positions will be comparable to positions obtained by previous MWRA monitoring activities as well as by other researchers that have used or are using dGPS at these stations. The station locations are targets and sampling for flounder and lobster will be conducted within 300 m of the targets but will ultimately be based on the availability of individual organisms. The deployment of mussels will be conducted within 15 m of the targets as determined by Normandeau's dGPS.

Completeness
For all navigation data, 100% completeness has been defined as the QAPP requirement. Normandeau's differential GPS (dGPS) navigation system will be used to acquire navigation data for mussel surveys. The shipboard navigation system on the fishing vessel *Harvest Moon* will be used for flounder surveys. For lobster surveys, the commercial vessel's navigation system will be used, and coordinates will be verified with a handheld GPS unit operated by the Normandeau field technician. The initial and final coordinates of each flounder trawl and the actual coordinates of each lobster pot will be hand recorded on field logsheets. The location of the mussel collections and array deployments will be hand recorded onto the Station Log. Depth measurements will be recorded at each station.

B5.1.2 *Flounder Survey*

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.

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 7, along with corrective actions.

Table 7. Data Quality Objectives (DQOs) for Physical Measurements of Flounder and Lobster

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

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.

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.

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.

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 Fish and Shellfish Project Area 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.

B5.1.3 *Lobster Survey*

Precision

The precision of lobster carapace length and lobster weight measurements will be monitored through the re-measurement of at least 10% of the specimens collected at each sampling site. DQOs for precision of physical measurements are defined in Table 7. If agreement between the length or weight measurements meets the DQOs, measurements will continue. If measurements or weights differ by more than the QAPP criteria, the cause will be identified and all specimens measured since the last acceptable precision measurement will be re-measured or re-weighed.

Accuracy

Traditional measures of accuracy do not directly apply to lobster collection procedures. However, accuracy measures do apply to the physical measurements. The accuracy of the Ohaus balance (Model C-11) used for weight determination is 1 g. The accuracy of the calipers is 0.02 mm.

Representativeness

Representativeness is primarily addressed through sample design. The sampling sites represent previously sampled locations and represent the population of lobsters that may be found within Boston Harbor and Offshore. Representativeness will also be ensured by proper handling, storage, and analysis of samples, as defined in this QAPP, so that the lobster tissues collected and analyzed reflect the conditions at the site locations.

Comparability

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

Completeness

The sampling objective is to obtain 15 commercially harvestable specimens representative of their location. The completeness goal for lobster sampling is 100%. However, if every reasonable effort to acquire the required number of lobsters has been made, and lobsters were not available for collection, the 100% completeness goal may be waived.

B5.1.4 *Mussel Bioaccumulation Survey*

Precision

There are no DQOs for the precision of mussel physical measurements.

Accuracy

Traditional measures of accuracy do not apply to mussel collection procedures. Calipers will be used to ensure that the mussels used in the survey are approximately six centimeters.

Representativeness

The sampling sites represent previously sampled locations and are representative of the expected short-term bioaccumulation conditions for mussels. Representativeness will also be ensured by proper

handling, storage, and analysis of samples, as defined in this QAPP, so that the mussel tissues collected and analyzed reflect the conditions at the site locations.

Comparability
The deployment and retrieval of caged mussels for short-term bioaccumulation is identical to the design of previous surveys. Mussels from the established reference site, Stover's Point, ME, will be relocated to different environments (station locations).

Completeness
Completeness goal for mussel collection is 100% (after the 60-day deployment), which should be achievable with the 15% additional contingency deployments (planning for potential loss of some mussel arrays). Large numbers of mussels are deployed in arrays, which should provide sufficient mussels for chemical analyses. An early 40-day retrieval is conducted to ensure sufficient tissue is available for each site (see Section 8 for further details on mussel deployment and collection plans).

B5.2 Flounder Histological Analysis and Age Determination

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

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 otoliths and scales will be read by NMFS or Normandeau scientists who are experienced in aging winter flounder.

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.

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).

Completeness
Tissues and otoliths or 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.

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 Ms. Thornton at NMFS for the age analysis task.

Under HOM9, there are no analytical laboratory instruments covered by this QAPP.

B7. INSTRUMENT/EQUIPMENT CALIBRATION AND FREQUENCY

B7.1 Navigation Equipment

Typically, the Captains of the boats being used will provide GPS navigation systems for the flounder and lobster surveys and will ensure that the instrumentation is well maintained. However, because the primary business of the boats used for this project is commercial fishing and not scientific collection, maintenance schedules are irregular. Therefore, Normandeau's field technicians and Dr. Moore will use portable GPS

units as a back-up for the shipboard navigation systems. 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. Normandeau's research vessels, equipped with GPS units that are maintained to the manufacturer's specifications, will be used for the mussel deployment and collections.

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 HOM9, there are no analytical laboratory instruments covered by this QAPP.

B8. INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES

For HOM9, critical supplies for field activities will be the responsibility of the Chief Scientist and the boat Captain (Table 8).

Table 8. 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
Field logs, COC forms, sample labels	Visually inspected	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. 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

Custody of field data will be the responsibility of the Chief Scientist during the field activity. Field data will be will be hand-recorded on the field log for flounder, lobster, and mussel surveys (Figures 5 to 8). For the flounder surveys, field data will include navigation data, survey ID, date, time, trawl number, vessel position at start and completion of each sampling event, and measurements and condition of flounders. Data recorded for the lobster surveys will include navigation data, survey ID, date, time, and position of each lobster pot, as well as measurements and condition of lobsters. And for mussel surveys, navigation data, survey ID, date, time, and location and condition of arrays. The Normandeau Field Manager must receive a complete copy of the survey log for each survey.

WHOI, NMFS, and EnviroSystems will collect data in the laboratory under this contract. The data files will remain in the custody of the analysts until all data have gone through the appropriate QA/QC checks. The custodian of data for histologic analyses will be Dr. Michael Moore, of WHOI. He will be responsible for data collection, entry, and 100% verification of all hand-entered data. The electronic histology data will be transferred to Normandeau's data manager, Mr. Eric Nestler. The EnviroSystems laboratory manager will be responsible for data collected during the processing of flounder, lobster, or mussels. The data from EnviroSystems will be transferred on paper data sheets to Normandeau for data entry and inclusion into the team's project database.

Data submissions (both hard copy and electronic) will be logged in upon receipt at Normandeau by the data manager and a copy of the login will be maintained in the project files.

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 Data Entry and Processing

Flounder survey data collected on field logs will be entered by Dr. Moore. Verification will be provided though a 100% quality check of the electronic data against the field logs.

Field collection logs for the lobster and mussel surveys will be delivered to the Normandeau data manager for data entry. Normandeau staff will enter data using the KeyesPunch™ software application, which employs automated controls and data verification. Formats designed to comply with rules of the EM&MS database will be used to constrain data entry, and data verification will be provided through double data entry. These features will ensure that any entry errors are caught and corrected as the operator keys the data.

Normandeau's FTP site will be used for file transfers of large files. Recipients will be notified of the submittal by email. Files will be available on the FTP site for seven days, after which Normandeau may remove them from the site.

B10.3 Data Reduction

Data reduction is the process of converting raw numbers into data that can be summarized in tables, displayed graphically, or compared statistically. Two stages in the data management process will involve data reduction: (1) before submission of data to MWRA, and (2) before inclusion of results in the annual report. Data reduction will be performed by the Normandeau data management team using SAS software.

Certain data from the flounder survey will require manipulation before being submitted to MWRA for entry into the project database. Bottom depth values will be recorded at the start and end of each trawl. Depth values for individual trawls and stations will be derived from these values as follows: DEPTH_TO_BOTTOM in the TRAWL table (see Appendix C) will be computed as the mean of start and end depths for each trawl. DEPTH_TO_BOTTOM in the STATION table will be computed as the mean of all DEPTH_TO_BOTTOM values for each station in the TRAWL table.

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. Also, 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.

B10.4 Data Set Structure

Electronic Data Deliverables will be prepared by Normandeau in a structure and format that complies with the MWRA database rules. Specifications for data sets and a description of sample identification for tissue chemistry composites are provided in Appendix C.

B10.5 Project Database Codes

Because the studies included in the Fish and Shellfish Monitoring Program deal with individual species and sampling protocols, it is anticipated that there will be limited need to establish new codes for the database. Observation of previously unseen abnormalities on flounder or lobsters could require new codes. If these, or other unforeseen situations, arise during the course of the project, Normandeau's Data Manager will create a provisional code, if necessary so that data can continue to be recorded until the new code can be submitted to and approved by MWRA. The current codes are listed in Tables 8 through 11.

B10.6 Data Submittal to MWRA

Prior to submittal to MWRA, all data will receive a quality assurance review by Normandeau during which SAS software will be used for logical error checks and to check for violations of EM&MS database constraints and business rules. Any issues will be corrected in the data files. Any irresolvable issues in the data files identified by quality control checks (for example, stations more than specified distance from target) will also be submitted to MWRA with the data deliverable.

Electronic data submissions will be made by Normandeau's data manager using MWRA's HOML web application.

B10.7 Data Report Quality Control Checks

Range checks will be performed on the parameters given in Table 13. These checks will be done by MWRA and reviewed by Normandeau and WHOI as part of the data reporting process (see section A.9.5).

Table 9 Data Qualifiers

Qualifier	Description	Value Reported?
e	Results not reported, value given is NULL, see comments field	No
w	This datum should be used with caution, see comment field	Yes
s	Suspect/Invalid. Not fit for use	Yes
j	Estimated value	Yes

Table 10. Morphological Parameters and Database Codes for Fish and Shellfish Monitoring.

SPECIES	PARAM_CODE	DESCR	UNIT_CODE	METH_CODE
Homarus americanus	CARAP_LEN	Carapace Length	mm	CLM
Homarus americanus	SEX	Gender		VISUAL
Homarus americanus	WEIGHT	Wet Weight of Organism	g	LWEIGHT
Pseudopleuronectes americanus	AGE	Chronological age of specimen	y	OTOLITH or SCALE
Pseudopleuronectes americanus	SEX	Gender		VISUAL
Pseudopleuronectes americanus	STAN_LEN	Standard length of a fish. From upper jaw tip to posterior end of the hypural bone.	mm	SLM
Pseudopleuronectes americanus	TOTAL_LEN	Total Length	mm	TLM
Pseudopleuronectes americanus	WEIGHT	Wet Weight of Organism	g	PWEIGHT

Table 11. Histopathological Parameters and Database Codes for Fish and Shellfish Monitoring.

SPEC_CODE	DESCR	FRACTION_CODE	PARAM_CODE	DESCR
8857041504	*Pseudopleuronectes americanus*	LIVER_SECTION	BILE_DUCT_PROTOZOAN	Presence (1) or absence (0) of flounder liver bile duct unicellular parasites in histology sections
8857041504	*Pseudopleuronectes americanus*	LIVER_SECTION	BALLOONS	Apoptopic lesion prevalence, rated on a scale from 0-4.
8857041504	*Pseudopleuronectes americanus*	LIVER_SECTION	BIL_PROLIF	Biliary proliferation
8857041504	*Pseudopleuronectes americanus*	LIVER_SECTION	CENTRO_HV	Centrotubular hydropic vacuolation
8857041504	*Pseudopleuronectes americanus*	LIVER_SECTION	FOCAL_HV	Focal hydropic vacuolation
8857041504	*Pseudopleuronectes americanus*	LIVER_SECTION	LIVER_FLUKES	Coded score (0-4) for presence of flounder liver flukes in histology sections
8857041504	*Pseudopleuronectes americanus*	LIVER_SECTION	MACROPHAGE	Macrophage aggregation, rated on a scale from 0-4.
8857041504	*Pseudopleuronectes americanus*	LIVER_SECTION	NEOPLASM	Neoplasia prevalence, rated on a scale from 0-4.
8857041504	*Pseudopleuronectes americanus*	LIVER_SECTION	TUBULAR_HV	Tubular hydropic vacuolation
8857041504	*Pseudopleuronectes americanus*	WHOLE_BODY	FIN_ROT	Fin rot score
8857041504	*Pseudopleuronectes americanus*	WHOLE_BODY	GROSS_LIV_LESIONS	Gross lesions visible on whole flounder liver
8857041504	*Pseudopleuronectes americanus*	WHOLE_BODY	ULCER	Flounder skin ulcer
8857041504	*Pseudopleuronectes americanus*	WHOLE_BODY	LYMPHO	Lymphocystis
8857041504	*Pseudopleuronectes americanus*	WHOLE_BODY	BENT_FIN	Bent fin ray
8857041504	*Pseudopleuronectes americanus*	WHOLE_BODY	NET_DAMAGE	Net damage
8857041504	*Pseudopleuronectes americanus*	WHOLE_BODY	LIVER_COL	Liver color
6181010201	*Homarus americanus*	WHOLE_BODY	BLACK_GILL	Black gill disease
6181010201	*Homarus americanus*	WHOLE_BODY	EXT_TUMORS	External tumors
6181010201	*Homarus americanus*	WHOLE_BODY	PARASITES	Parasite prevalence, rated on a scale from 0-4.
6181010201	*Homarus americanus*	WHOLE_BODY	SHELL_EROS	Shell erosion

Table 12. General Database Codes for Fish and Shellfish Monitoring.

Field Name	Code	Description
ANAL_LAB_ID	WHO4	Woods Hole Oceanographic Institution – M. Moore
FRACTION_CODE	FILLET	Fillet of fish (edible tissue)
FRACTION_CODE	HEPATOPANC	Hepatopancreas
FRACTION_CODE	INDIVIDUAL	Measurement was made on an individual animal
FRACTION_CODE	LIVER	Analysis done on liver only
FRACTION_CODE	LIVER_SECTION	Analysis done on section of liver
FRACTION_CODE	MEAT	Edible meat from lobster (tail and claw)
FRACTION_CODE	SOFT_TISSUE	Entirety of organisms soft tissue
FRACTION_CODE	WHOLE_BODY	Entire body or part of body described at PARAM_CODE level
GEAR_CODE	ARRAY	Mussel deployment array
GEAR_CODE	OTT	Otter trawl tow
GEAR_CODE	TRAP	Lobster trap
INSTR_CODE	BAL	Balance
INSTR_CODE	MICR	Microscope
INSTR_CODE	RULER	Measurement by ruler
MATRIX_CODE	5507010101	Mytilus edulis
MATRIX_CODE	5507010101_C	Composite of *Mytilus edulis*
MATRIX_CODE	6181010201	*Homarus americanus*
MATRIX_CODE	6181010201_C	Composite of *Homarus americanus*
MATRIX_CODE	8857041504	*Pseudopleuronectes americanus*
MATRIX_CODE	8857041504_C	Composite of *Pseudopleuronectes americanus*
METH_CODE	CLM	Caliper measurement as mentioned in CW/QAPP for fish and shellfish monitoring, sec.11.3. ENSR 1997
METH_CODE	FSF98	Method for pathology parameters described in fish and shellfish QAPP
METH_CODE	LWEIGHT	Lobster weight to the nearest gram using conventional scale (model 70-2030)
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	OTOLITH	Aging by otolith
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
UNIT_CODE	PCT	PERCENT
UNIT_CODE	y	years

Table 13. Range Checks for Flounder and Lobster Monitoring.

Parameter	Flounder	Lobster
Length	Range check against longest and shortest flounder from previously acceptable data. Specimens outside this range will be flagged.	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.	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.	NA
Liver Histopathology	Plot prevalence by station to ensure that no obvious errors, such as reporting tumors at a station where none were seen.	NA

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 HOM9 fish and shellfish monitoring. These activities will be overseen by the Normandeau Project QA Officer, Mr. Robert Hasevlat.

C1.1 Planned Assessments

Each field survey plan will include a checklist for required supplies and equipment, including items required for the health and safety of field personnel. In preparation for each survey, the chief scientist will be responsible for reviewing and completing this checklist. An example Field Safety and Equipment Checklist is shown in Figure 11.

The planned project audits are included in Table 14. These survey assessments will address field, laboratory, and data management activities. Examples of field and laboratory audit checklists are provided in Tables 15 and 16. Assessment of data management activities will be accomplished through the quality audits of data deliverables, resulting in a Quality Assurance Statement signed by Mr. Hasevlat, the Normandeau QA Director, and submitted as part of each data deliverable to MWRA.

C1.2 Assessment Findings and Corrective Action Responses

The assessment findings and recommended corrective actions for the planned assessment tasks listed in Table 14 will be documented by the project team member responsible for performing the assessment. Additionally, the recommended corrective actions will be followed up by the project team member responsible for the assessment to ensure that procedures have come into compliance with this QAPP.

Corrective actions may result from planned audits or from unanticipated events that occur during the course of the project. Significant events that result in deviations from this QAPP will be recorded through Normandeau's "Extraordinary Event Non-Conformity" (EENC) reporting process. The appropriate corrective actions to address any such events will be assessed by Mr. Hasevlat in consultation with Normandeau Project Manager, Ann Pembroke, and with MWRA. Mr. Hasevlat will generate and/or review all corrective actions required during the project and monitor their effectiveness in meeting project quality objectives. Ms. Pembroke will review these issues on a monthly basis, but the QA Director will bring serious issues to Ms. Pembroke's attention immediately. Ms. Pembroke will report corrective actions to MWRA in quarterly QA/QC Corrective Action Logs.

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).

<u>Field Corrective Action</u>
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 Fish and Shellfish Project Area Manager, Normandeau Program Manager, and Normandeau 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 Fish and Shellfish Project Area Manager will be notified.

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 Normandeau Program Manager and Project QA Officer will be notified. The Normandeau Program Manager will communicate with the MWRA Fish and Shellfish Monitoring Project Area Manager and other members of the project team, as necessary. The MWRA Fish and Shellfish 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 Normandeau Program Manager, who will determine the action to be taken and inform the appropriate personnel.

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 Normandeau Program Manager will be responsible for informing the appropriate personnel, including the MWRA Fish and Shellfish Monitoring Project Area Manager.

Table 14. Planned Project Assessments

Assessment Type	Frequency	Internal or External	Organization Performing Assessment	Person(s) Responsible for Performing Assessment (Title and Organizational Affiliation)	Person(s) Responsible for Responding to Assessment Findings (Title and Organizational Affiliation)	Person(s) Responsible for Identifying and Implementing Corrective Actions (CA) (Title and Organizational Affiliation)	Person(s) Responsible for Monitoring Effectiveness of CA (Title and Organizational Affiliation)
Flounder Survey	Annual	Internal	Normandeau	Robert Hasevlat QA Director	Michael Moore Chief Scientist/WHOI	Michael Moore	Robert Hasevlat
Lobster Survey	Once, in 2015	Internal	Normandeau	Robert Hasevlat QA Director	Paul Geoghegan/ Survey Manager/Normandeau	Paul Geoghegan	Robert Hasevlat
Mussel Survey	Once, in 2015	Internal	Normandeau	Robert Hasevlat QA Director	Erik Fel'Dotto Field Manager/Normandeau	Erik Fel'Dotto	Robert Hasevlat

Figure 11. Field Safety and Equipment 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:

_____ _____

_____ _____

_____ _____

_____ _____

_____ _____

_____ _____

Table 15. Example of Field Audit 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:**

Table 16. 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:**

C2. REPORTS TO MANAGEMENT

QA reports to management include formal reports (e.g., this QAPP), logs (e.g., Corrective Action Logs, EENCs), and verbal or email communication with MWRA.

Reports involving corrective actions will be prepared by the Normandeau Project QA Officer and submitted on an as-needed basis to the Normandeau Program Manager. Such reports will document any problems identified during the sampling and analysis programs and the corrective measures taken in response. These 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 sections B10 and D2 of 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, (2) age analysis data provided by NMFS or Normandeau, and (3) data collected by EnviroSystems. Review processes for these data are described in sections B10 and D2 of this QAPP.

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 of this QAPP.

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 fish and shellfish monitoring tasks 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

Following review of the data, all suspect data will be reported, but flagged with a qualifier. These data may not be used in calculations or data summaries without the approval of MWRA. 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 Normandeau prior to the electronic submission to MWRA. Data review will include methods such as scatter plots, univariate and multivariate analyses, and range checks to identify suspect values. Routine system back-ups are performed daily. Data provided electronically to facilitate data handling will be verified against the hard copy data. Additional review of the data by Normandeau and Dr. Moore 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. Detailed description of data management and review is provided in section B10 of this QAPP.

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 HOM9 fish and shellfish 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 Data Quality Objectives (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, and by Normandeau.

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 HOM9 Fish and Shellfish Monitoring Program is 100% of

planned samples to be collected and analyzed. The Normandeau 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 Normandeau 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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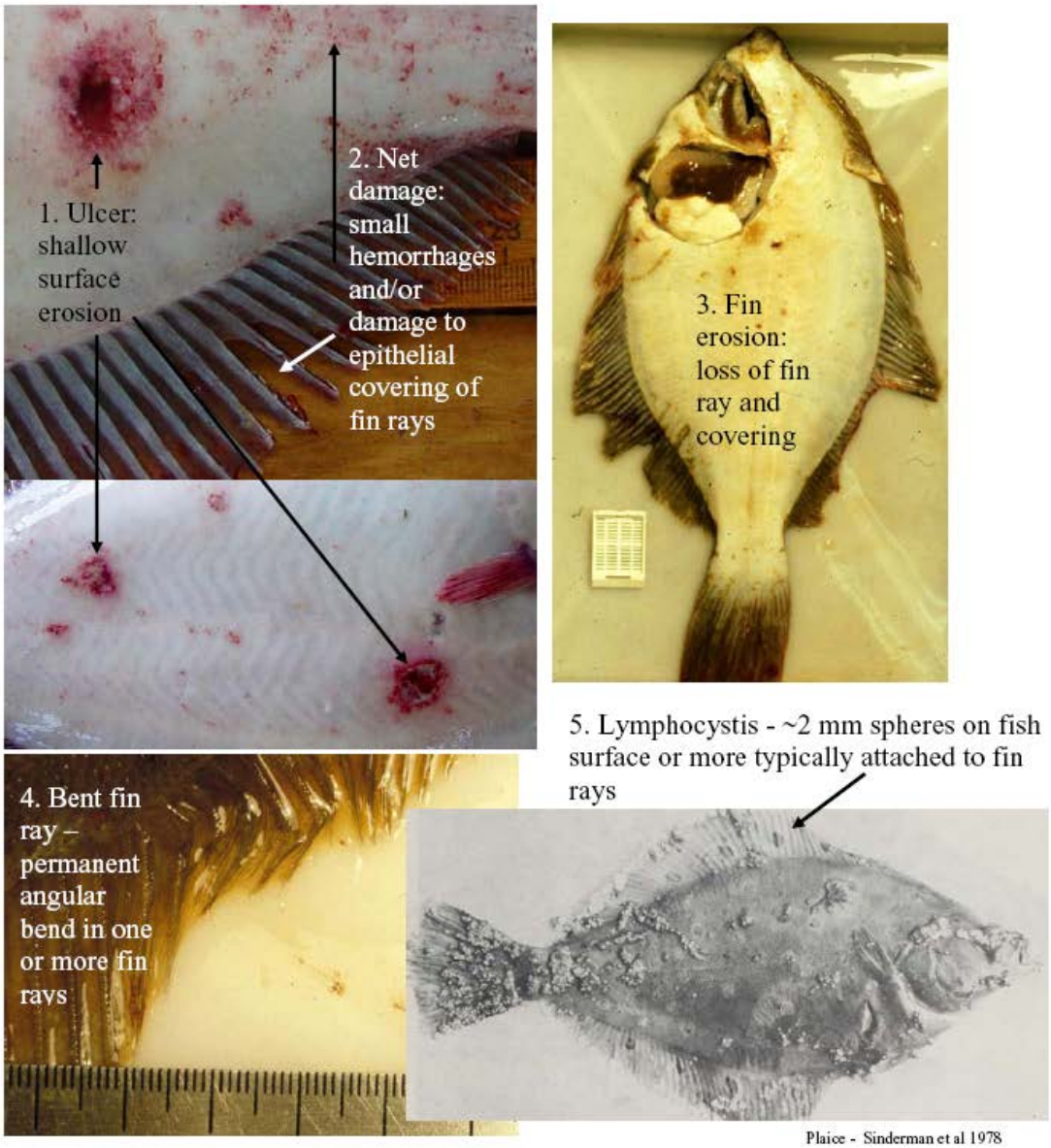
Appendix A
Guidance for Recording External Lesions in Flounder

SCORING EXTERNAL LESIONS ON WINTER FLOUNDER



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.

Appendix B
Flounder Tissue Resection SOP

Standard Operating Protocol for Dissection of Winter Flounder following FF151 Flounder Survey for Normandeau/ EnviroSystems

M Moore, Woods Hole Oceanographic Institution.
508 289 3228 tel 508 989 3575 cell mmoore@whoi.edu

Flounder will have been measured, weighed, scale samples taken and fish observed (and sampled if need be) for external lesions before they leave the vessel.

Materials provided from vessel with the first shipment of fish:

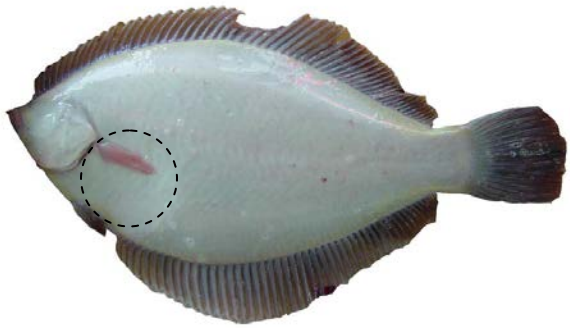
Cooler with ice and individually bagged and tagged flounder
Histology cassettes
Cassette marking pencil
4 screw top jars of buffered formalin (one per station)
Data sheet with field data/ Chain of custody form
Titanium forceps and blade to dissect out liver.
Rubber bands to ensure cassettes do not open in transit. Three cassettes can be banded with one band.

Material needed in lab:

Ceramic scissors to open body wall
Titanium knife to remove fillets
Containers for liver and fillet chemistry samples
Balance to weigh samples taken for chemistry
Digital camera

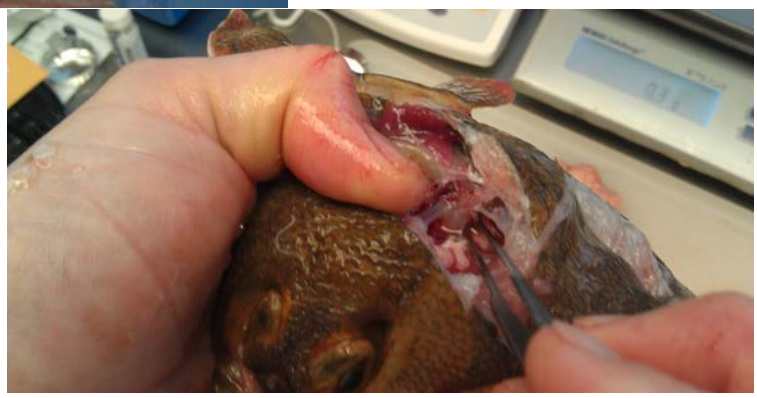
Procedure

Clean all instruments
Open blind side of visceral cavity – see dotted line below

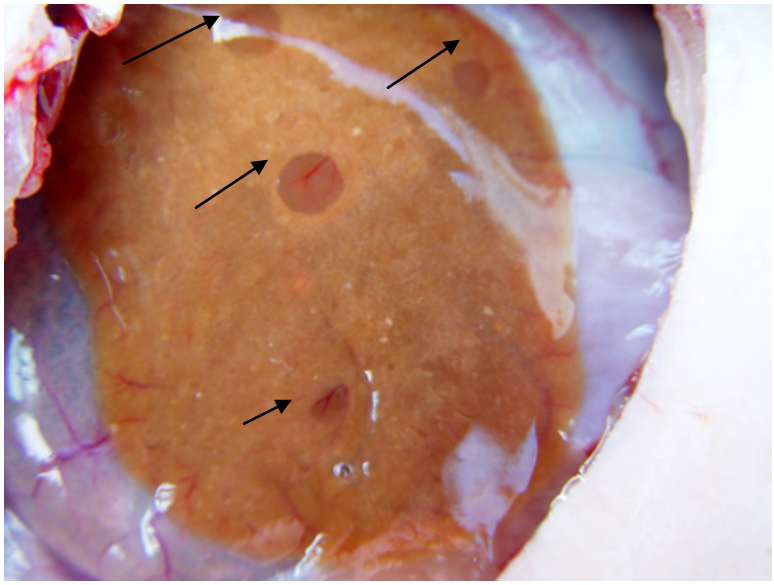


Otoliths will then be removed from either side of the brainstem after exposure with one or other of the two cuts illustrated below (2014 and 2015, only).

Incision options to expose otoliths.
Left: eyed side.
Top: blind side. Bottom: location of otoliths by brain stem.



Examine liver surfaces and cut section (~every 3mm) surfaces for gross lesions.
Enter a score in the data sheet in the Gross liver lesion column.
0 = None
1 = one circular discoloration – will be either clear and unpigmented or creamy white, as compared to the body of the liver.
2 = Two lesions
3 = Three lesions
4 = Four or more lesions



Four clear lesions are illustrated here. Score = 4.

If in doubt, take a close up, in focus photograph of the lesion with a label showing the fish ID and submit with samples to WHOI for confirmation.

Record the liver color Y:Yellow, YB:Y Brown, B: Brown, DB: Dark Brown

Confirm gender on data sheet by examining gonads (these fish can be sexed by palpating the blind side peduncle, but it is always good to examine the gonads to confirm.
White and milky = male,
Pink and granular= female
Note any discrepancy on data sheet.

Remove liver, avoiding gall bladder rupture.
Remove three small equidistant transverse slices of liver (c. 10x10x3mm) and place in a labeled cassette. Close cassette and place in jar of formalin. Ideally the formalin jar remains in a fume hood whenever it is open. Once complete seal the jar lids with parafilm and packing tape.

Put rest of liver in chemistry pool. Each of 3 pools consists of tissue from 5 fish.
Remove filets and pool in same way.

When completed, check data sheet for completeness, and send a copy of that plus the jars of formalin fixed liver slices in formalin, coolers, and supply/ tool box to:
Michael Moore
Mailstop 50
MRF 221
WHOI
Woods Hole MA 02543

Material Safety Data Sheet

10% Buffered formalin

ACC# 41129

Section 1 - Chemical Product and Company Identification

MSDS Name: 10% Buffered formalin
Catalog Numbers: SF99-20, SF99-4
Synonyms: None.
Company Identification:
Fisher Scientific
1 Reagent Lane
Fair Lawn, NJ 07410
For information, call: 201-796-7100
Emergency Number: 201-796-7100
For CHEMTREC assistance, call: 800-424-9300
For International CHEMTREC assistance, call: 703-527-3887

Section 2 - Composition, Information on Ingredients

CAS#	Chemical Name	Percent	EINECS/ELINCS
50-00-0	Formaldehyde	3.9-4.0	200-001-8
67-56-1	Methyl alcohol	2	200-659-6
127-09-3	Sodium acetate	1.2-2.0	204-823-8
7732-18-5	Water	Balance	231-791-2

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Appearance: not available liquid. Flash Point: > 194 deg F.
Warning! Contains formaldehyde which can cause cancer. Causes eye, skin, and respiratory tract irritation. May cause allergic respiratory and skin reaction. May be harmful if swallowed or absorbed through the skin. May cause central nervous system depression. This substance has caused adverse reproductive and fetal effects in animals.
Target Organs: Central nervous system, lungs, respiratory system, skin.

Potential Health Effects

Eye: Causes eye irritation.
Skin: Causes skin irritation. May cause skin sensitization, an allergic reaction, which becomes evident upon re-exposure to this material.
Ingestion: Cannot be made non-poisonous. May cause central nervous system depression, kidney damage, and liver damage. Causes gastrointestinal irritation with nausea, vomiting and diarrhea.
Inhalation: Causes respiratory tract irritation. May cause allergic respiratory reaction.
Chronic: Contains formaldehyde which can cause cancer in humans. There is sufficient evidence that formaldehyde causes nasopharyngeal cancer in humans, a rare cancer in developed countries. There is limited evidence that formaldehyde causes cancer of the nasal cavity and paranasal sinuses and strong but not sufficient evidence for leukemia.

Section 4 - First Aid Measures

Eyes: Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Get medical aid.

Skin: Get medical aid. Flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes.
Ingestion: Do not induce vomiting. If victim is conscious and alert, give 2-4 cupfuls of milk or water. Never give anything by mouth to an unconscious person. Get medical aid.
Inhalation: Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid.
Notes to Physician: Treat symptomatically and supportively.

Section 5 - Fire Fighting Measures

General Information: As in any fire, wear a self-contained breathing apparatus in pressure-demand, MSHA/NIOSH (approved or equivalent), and full protective gear. During a fire, irritating and highly toxic gases may be generated by thermal decomposition or combustion.
Extinguishing Media: For small fires, use dry chemical, carbon dioxide, water spray or alcohol-resistant foam. Cool containers with flooding quantities of water until well after fire is out.
Flash Point: > 194e deg F (> 90.00 deg C)
Autoignition Temperature: Not applicable.
Explosion Limits, Lower: Not available.
Upper: Not available.
NFPA Rating: (estimated) Health: 2; Flammability: 1; Instability: 0

Section 6 - Accidental Release Measures

General Information: Use proper personal protective equipment as indicated in Section 8.
Spills/Leaks: Absorb spill with inert material (e.g. vermiculite, sand or earth), then place in suitable container. Clean up spills immediately, observing precautions in the Protective Equipment section. Provide ventilation.

Section 7 - Handling and Storage

Handling: Wash thoroughly after handling. Wash hands before eating. Remove contaminated clothing and wash before reuse. Do not get in eyes, on skin, or on clothing. Keep container tightly closed. Do not ingest or inhale. Use only with adequate ventilation.
Storage: Store in a tightly closed container. Store in a cool, dry, well-ventilated area away from incompatible substances.

Section 8 - Exposure Controls, Personal Protection

Engineering Controls: Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower. Use adequate general or local exhaust ventilation to keep airborne concentrations below the permissible exposure limits. See 29CFR 1910.1048 for regulatory requirements pertaining to all occupational exposures to formaldehyde, i.e., from formaldehyde gas, its solutions, and materials that release formaldehyde.

Exposure Limits

Chemical Name	ACGIH	NIOSH	OSHA - Final PELs
Formaldehyde	0.3 ppm Ceiling	0.016 ppm TWA 20 ppm IDLH	0.75 ppm TWA; 2 ppm STEL; 0.5 ppm Action Level (Irritant and potential cancer hazard - see 29 CFR 1910.1048)
Methyl alcohol	200 ppm TWA; 250 ppm STEL; Skin - potential significant contribution to overall exposure by the cutaneous route	200 ppm TWA; 260 mg/m3 TWA 6000 ppm IDLH	200 ppm TWA; 260 mg/m3 TWA
Sodium acetate	none listed	none listed	none listed
Water	none listed	none listed	none listed

OSHA Vacated PELs: Formaldehyde: 3 ppm TWA (unless specified in 1910.1048) Methyl alcohol: 200 ppm TWA; 260 mg/m3 TWA Sodium acetate: No OSHA Vacated PELs are listed for this chemical. Water: No OSHA Vacated PELs are listed for this chemical.

Personal Protective Equipment

Eyes: Wear chemical splash goggles.
Skin: Wear appropriate protective gloves to prevent skin exposure.
Clothing: Wear appropriate protective clothing to prevent skin exposure.
Respirators: Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced.

Section 9 - Physical and Chemical Properties

Physical State: Liquid
Appearance: not available
Odor: none reported
pH: Not available.
Vapor Pressure: Not available.
Vapor Density: Not available.

Evaporation Rate: Not available.
Viscosity: Not available.
Boiling Point: Not available.
Freezing/Melting Point: Not available.
Decomposition Temperature: Not available.
Solubility: Soluble in water.
Specific Gravity/Density: Not available.
Molecular Formula: Mixture
Molecular Weight: Not applicable.

Section 10 - Stability and Reactivity

Chemical Stability: Stable under normal temperatures and pressures.
Conditions to Avoid: Excess heat, confined spaces.
Incompatibilities with Other Materials: Strong oxidizing agents.
Hazardous Decomposition Products: Irritating and toxic gases.
Hazardous Polymerization: Has not been reported

Section 11 - Toxicological Information

RTECS#:
CAS# 50-00-0: LP8925000
CAS# 67-56-1: PC1400000
CAS# 127-09-3: AJ4300010
CAS# 7732-18-5: ZC0110000
LD50/LC50:
CAS# 50-00-0:
Draize test, rabbit, eye: 750 ug/24H Severe;
Draize test, rabbit, eye: 750 ug Severe;
Draize test, rabbit, eye: 10 mg Severe;
Draize test, rabbit, eye: 37% Severe;
Draize test, rabbit, skin: 2 mg/24H Severe;
Draize test, rabbit, skin: 50 mg/24H Moderate;
Inhalation, mouse: LC50 = 454 mg/m3/4H;
Inhalation, mouse: LC50 = 505 mg/m3/2H;
Inhalation, rat: LC50 = 203 mg/m3;
Inhalation, rat: LC50 = 578 mg/m3/2H;
Inhalation, rat: LC50 = 250 ppm/2H;
Oral, mouse: LD50 = 42 mg/kg;
Oral, mouse: LD50
CAS# 67-56-1:
Draize test, rabbit, eye: 40 mg Moderate;
Draize test, rabbit, eye: 100 mg/24H Moderate;
Draize test, rabbit, skin: 20 mg/24H Moderate;

Inhalation, rabbit: LC50 = 81000 mg/m3/14H;
Inhalation, rat: LC50 = 64000 ppm/4H;
Oral, mouse: LD50 = 7300 mg/kg;
Oral, rabbit: LD50 = 14200 mg/kg;
Oral, rat: LD50 = 5600 mg/kg;
Skin, rabbit: LD50 = 15800 mg/kg;
.

CAS# 127-09-3:
Draize test, rabbit, eye: 10 mg Mild;
Draize test, rabbit, skin: 500 mg/24H Mild;
Inhalation, rat: LC50 = >30 gm/m3/1H;
Oral, mouse: LD50 = 6891 mg/kg;
Oral, rat: LD50 = 3530 mg/kg;
Skin, rabbit: LD50 = >10 gm/kg;
.

CAS# 7732-18-5:
Oral, rat: LD50 = >90 mL/kg;
.

Carcinogenicity:
CAS# 50-00-0:

- **ACGIH:**

  A2 - Suspected Human Carcinogen

- **California:**

  carcinogen, initial date 1/1/88 (gas)

- **NTP:**

  Suspect carcinogen

- **IARC:**

  Group 1 carcinogen

CAS# 67-56-1: Not listed by ACGIH, IARC, NTP, or CA Prop 65.
CAS# 127-09-3: Not listed by ACGIH, IARC, NTP, or CA Prop 65.
CAS# 7732-18-5: Not listed by ACGIH, IARC, NTP, or CA Prop 65.

Epidemiology: In June 2004 an expert IARC group determined that there is now sufficient evidence that formaldehyde causes nasopharyngeal cancer in humans, a rare cancer in developed countries.
Teratogenicity: Formaldehyde effects on Newborn: behavioral, ihl-rat TCLo=50 ug/m3/4H; biochemical/metabolic and reduced weight gain, ihl-rat TCLo=12 ug/m3/24H. Embryo or Fetus: cytological changes, ihl-rat TCLo=1 mg/m3/24H; stunted fetus and death, ipr-mouse TDLo=240 mg/kg. Specific Developmental Abnormalities: craniofacial and musculoskeletal,

ipr-mouse TDLo=240 mg/kg.
Reproductive Effects: Formaldehyde effects on Fertility: male index, itt-rat TDLo=400 mg/kg; post- implantation mortality, ims-mouse TDLo=259 mg/kg. Paternal Effects: spermatogenesis, orl-rat TDLo=200 mg/kg; testes/sperm duct/epididymis, ipr-rat TDLo=80 mg/kg.
Mutagenicity: Formaldehyde DNA Damage: human fibroblast 100 umol/L DNA Inhibition: human cell types 210 umol/L. Unscheduled DNA Synthesis: rat cell types 50 umol/L. Gene Mutation in Mammalian Cells: human lymphocyte 130 umol/L.
Neurotoxicity: No information available.
Other Studies:

Section 12 - Ecological Information

No information available.

Section 13 - Disposal Considerations

Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. US EPA guidelines for the classification determination are listed in 40 CFR Parts 261.3. Additionally, waste generators must consult state and local hazardous waste regulations to ensure complete and accurate classification.
RCRA P-Series: None listed.
RCRA U-Series:
CAS# 50-00-0: waste number U122.
CAS# 67-56-1: waste number U154 (Ignitable waste).

Section 14 - Transport Information

	US DOT	Canada TDG
Shipping Name:	AVIATION REGULATED LIQUID, N.O.S.	AVIATION REGULATED LIQUID, N.O.S.
Hazard Class:	9	9
UN Number:	UN3334	UN3334
Packing Group:		

Section 15 - Regulatory Information

US FEDERAL

TSCA

CAS# 50-00-0 is listed on the TSCA inventory.
CAS# 67-56-1 is listed on the TSCA inventory.
CAS# 127-09-3 is listed on the TSCA inventory.
CAS# 7732-18-5 is listed on the TSCA inventory.

Health & Safety Reporting List

None of the chemicals are on the Health & Safety Reporting List.

Chemical Test Rules

None of the chemicals in this product are under a Chemical Test Rule.

Section 12b

None of the chemicals are listed under TSCA Section 12b.

TSCA Significant New Use Rule

None of the chemicals in this material have a SNUR under TSCA.

CERCLA Hazardous Substances and corresponding RQs

CAS# 50-00-0: 100 lb final RQ; 45.4 kg final RQ CAS# 67-56-1: 5000 lb final RQ; 2270 kg final RQ

SARA Section 302 Extremely Hazardous Substances

CAS# 50-00-0: 500 lb TPQ

SARA Codes

CAS # 50-00-0: immediate, delayed.
CAS # 67-56-1: immediate, fire.

Section 313

This material contains Formaldehyde (CAS# 50-00-0, 3.9-4.0%),which is subject to the reporting requirements of Section 313 of SARA Title III and 40 CFR Part 373.

This material contains Methyl alcohol (CAS# 67-56-1, 2%),which is subject to the reporting requirements of Section 313 of SARA Title III and 40 CFR Part 373.

Clean Air Act:

CAS# 50-00-0 is listed as a hazardous air pollutant (HAP).
CAS# 67-56-1 is listed as a hazardous air pollutant (HAP).

This material does not contain any Class 1 Ozone depletors.
This material does not contain any Class 2 Ozone depletors.

Clean Water Act:

CAS# 50-00-0 is listed as a Hazardous Substance under the CWA.
None of the chemicals in this product are listed as Priority Pollutants under the CWA.
None of the chemicals in this product are listed as Toxic Pollutants under the CWA.

OSHA:

CAS# 50-00-0 is considered highly hazardous by OSHA.

STATE

CAS# 50-00-0 can be found on the following state right to know lists: California, New Jersey, Pennsylvania, Minnesota, Massachusetts.

CAS# 67-56-1 can be found on the following state right to know lists: California, New Jersey, Pennsylvania, Minnesota, Massachusetts.

CAS# 127-09-3 is not present on state lists from CA, PA, MN, MA, FL, or NJ.
CAS# 7732-18-5 is not present on state lists from CA, PA, MN, MA, FL, or NJ.

California Prop 65
The following statement(s) is(are) made in order to comply with the California Safe Drinking Water Act:
WARNING: This product contains Formaldehyde, a chemical known to the state of California to cause cancer.
California No Significant Risk Level: CAS# 50-00-0: 40 æg/day NSRL

European / International Regulations

European Labeling in Accordance with EC Directives
Hazard Symbols:
T
Risk Phrases:
R 45 May cause cancer.

Safety Phrases:

WGK (Water Danger/Protection)
CAS# 50-00-0: 2
CAS# 67-56-1: 1
CAS# 127-09-3: 1
CAS# 7732-18-5: No information available.
Canada - DSL/NDSL
CAS# 50-00-0 is listed on Canada's DSL List.
CAS# 67-56-1 is listed on Canada's DSL List.
CAS# 127-09-3 is listed on Canada's DSL List.
CAS# 7732-18-5 is listed on Canada's DSL List.
Canada - WHMIS
This product has a WHMIS classification of B3, D1B, D2A.
This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations and the MSDS contains all of the information required by those regulations.
Canadian Ingredient Disclosure List
CAS# 50-00-0 is listed on the Canadian Ingredient Disclosure List.
CAS# 67-56-1 is listed on the Canadian Ingredient Disclosure List.

Section 16 - Additional Information

MSDS Creation Date: 7/12/1999
Revision #7 Date: 10/12/2005

The information above is believed to be accurate and represents the best information currently available to us. However, we make no warranty of merchantability or any other warranty, express or implied, with respect to such information, and we assume no liability resulting from its use. Users should make their own investigations to determine the suitability of the information for their particular purposes. In no event shall Fisher be liable for any claims, losses, or damages of any third party or for lost profits or any special, indirect, incidental, consequential or exemplary damages, howsoever arising, even if Fisher has been advised of the possibility of such damages.

Appendix C
Specifications for Data Sets

a. Flounder, lobster, or mussel survey data

Flounder, lobster, or mussel survey event (FL_EVENT, LO_EVENT, MY_EVENT)

Description	Field	Required Field	Data type & format
Identifier of sampling event (survey)	EVENT_ID	Y	alphanumeric, maximum 10 characters
Name of the event.	EVENT_NAME	Y	alphanumeric, maximum 100 characters
Platform name (e.g., vessel name or drifter serial #).	PLAT_NAME		alphanumeric, maximum 20 characters
Name of the scientist in charge of the event.	CHIEF_SCIENTIST		alphanumeric, maximum 20 characters
Comments on survey event, detailing any exceptions from standard procedures	COMMENTS		alphanumeric, maximum 150 characters

Flounder trawl (FL_TRAWL) (flounder surveys only)

Description	Field	Required Field	Data type & format
Identifier of sampling event (survey)	EVENT_ID	Y	alphanumeric, maximum 10 characters
Identifier for station.	STAT_ID	Y	alphanumeric, maximum 10 characters
Station arrival date (trawl date) and time (local time)	START_TRAWL_ DATE_TIME_LOCAL	Y	date
Beginning latitude measured at each station visit (decimal degrees)	BEG_LATITUDE	Y	number (7 decimal places)
Beginning longitude measured at each station visit (decimal degrees)	BEG_LONGITUDE	Y	number (7 decimal places)
Trawl end date and time (local time)	END_TRAWL_DATE _TIME_LOCAL	Y	date
Ending latitude measured at each station visit (decimal degrees)	END_LATITUDE		number (7 decimal places)
Ending longitude measured at each station visit (decimal degrees)	END_LONGITUDE		number (7 decimal places)
Depth to bottom in meters	DEPTH_TO_ BOTTOM		number (2 decimal places)
# of Winter flounder >300 mm	N_WF_300	Y	integer (6,0)
Comments detailing any exceptions from standard procedures	COMMENTS		alphanumeric, maximum 150 characters
Number of ghost traps caught in trawl net, which may reduce catch efficiency	GHOST_TRAPS		integer(2,0)

Flounder, lobster, or mussel station (FL_STATION, LO_STATION, MY_STATION)

Description	Field	Required Field	Data type & format
Identifier of sampling event (survey)	EVENT_ID	Y	alphanumeric, maximum 10 characters
Identifier for station.	STAT_ID	Y	alphanumeric, maximum 10 characters
Station arrival date and time (local time)	STAT_ARRIV _LOCAL	Y	date
Beginning latitude measured at each station visit (decimal degrees)	BEG_LATITUDE	Y	number (7 decimal places)
Beginning longitude measured at each station visit (decimal degrees)	BEG_LONGITUDE	Y	number (7 decimal places)
Ending latitude measured at each station visit (decimal degrees)	END_LATITUDE		number (7 decimal places)
Ending longitude measured at each station visit (decimal degrees)	END_LONGITUDE		number (7 decimal places)
Depth to bottom in meters	DEPTH_TO _BOTTOM	Y	number (2 decimal places)
How station location was determined (e.g., LORAN-C, line of sight, survey map, etc.).	NAVIGATION _CODE	Y	alphanumeric, maximum 20 characters
Estimated accuracy of navigation in meters.	NAV_QUAL	Y	alphanumeric, maximum 10 characters
Comments detailing any exceptions from standard procedures on this station visit	COMMENTS		alphanumeric, maximum 150 characters

Flounder or lobster sample (FL_SAMPLE, LO_SAMPLE)

Description	Field	Required Field	Data type & format
Identifier of sampling event (survey)	EVENT_ID	Y	alphanumeric, maximum 10 characters
Identifier for station.	STAT_ID	Y	alphanumeric, maximum 10 characters
Station arrival date and time (local time)	STAT_ARRIV_LOCAL	Y	date
Sample identifier (fish)	SAMPLE_ID	Y	alphanumeric, maximum 15 characters
Code for type of gear used to collect sample.	GEAR_CODE	Y	alphanumeric, maximum 12 characters
Comments for a given sample	COMMENTS		alphanumeric, maximum 150 characters

Mussel sample (MY_SAMPLE)

Description	Field	Required Field	Data type & format
Identifier of sampling event (survey)	EVENT_ID	Y	alphanumeric, maximum 10 characters
Identifier for station.	STAT_ID	Y	alphanumeric, maximum 10 characters
Station arrival date and time (local time)	STAT_ARRIV_LOCAL	Y	date
Depth of mussel cage from water surface, in m (use zero for shoreline mussel collection)	DEPTH	Y	number (2 decimal places)
Code for type of gear used to collect sample.	GEAR_CODE	Y	alphanumeric, maximum 12 characters
Sample identifier (fish)	SAMPLE_ID	Y	alphanumeric, maximum 15 characters
Station identifier for the site from which these mussels were originally collected (e.g. SP for Stover's Point)	REFSITE_STAT_ID		alphanumeric, maximum 10 characters
Comments for a given sample	COMMENTS		alphanumeric, maximum 150 characters

b. Flounder histology measurement data

Flounder morphology (FL_MORPH)

Description	Field	Required Field	Data type & format
Identifier of sampling event (survey)	EVENT_ID	Y	alphanumeric, maximum 10 characters
Identifier for station.	STAT_ID	Y	alphanumeric, maximum 10 characters
Sample identifier	SAMPLE_ID	Y	alphanumeric, maximum 15 characters
Total length (mm)	TOTAL_LEN	Y	floating point
Qualifier for TOTAL_LEN	TOTAL_LEN_QUAL		alphanumeric, maximum 4 characters
Standard length, from upper jaw tip to posterior end of the hypural bone.	STAN_LEN	Y	floating point
Qualifier for STAN_LEN	STAN_LEN_QUAL		alphanumeric, maximum 4 characters
Weight (g)	WEIGHT	Y	floating point

Description	Field	Required Field	Data type & format
Qualifier for WEIGHT	WEIGHT_QUAL		alphanumeric, maximum 4 characters
Gender	SEX	Y	alphanumeric, maximum 10 characters
Qualifier for SEX	SEX_QUAL		alphanumeric, maximum 4 characters
Age (y)	AGE	Y	floating point
Qualifier for AGE	AGE_QUAL		alphanumeric, maximum 4 characters
Flounder skin ulcer (0-4)	ULCER		alphanumeric, maximum 10 characters
Qualifier for ULCER	ULCER_QUAL		alphanumeric, maximum 4 characters
Net damage (0-4)	NET_DAMAGE		alphanumeric, maximum 10 characters
Qualifier for NET_DAMAGE	NET_DAMAGE _QUAL		alphanumeric, maximum 4 characters
Fin erosion (0-4)	FIN_ROT		alphanumeric, maximum 10 characters
Qualifier for FIN_ROT	FIN_ROT_QUAL		alphanumeric, maximum 4 characters
Bent fin ray (0-4)	BENT_FIN		alphanumeric, maximum 10 characters
Qualifier for BENT_FIN	BENT_FIN_QUAL		alphanumeric, maximum 4 characters
Lymphocystis (0-4)	LYMPHO		alphanumeric, maximum 10 characters
Qualifier for LYMPHO	LYMPHO_QUAL		alphanumeric, maximum 4 characters
IDs for photos of external lesions (0-many per fish)	PHOTO_RANGE		alphanumeric, maximum 10 characters
Code for lab that performed the analysis.	ANAL_LAB_ID		alphanumeric, maximum 4 characters
Number assigned by the laboratory to the sample.	LAB_SAMPLE_ID		alphanumeric, maximum 35 characters
Comments for a given sample (fish)	COMMENTS		alphanumeric, maximum 150 characters

Flounder photographs (FL_PHOTO)

Description	Field	Required Field	Data type & format
Identifier of sampling event (survey)	EVENT_ID	Y	alphanumeric, maximum 10 characters
Identifier for station.	STAT_ID	Y	alphanumeric, maximum 10 characters
Sample identifier	SAMPLE_ID	Y	alphanumeric, maximum 15 characters
Photo ID number	PHOTO_ID	Y	alphanumeric, maximum 10 characters
Comments for a given photo	COMMENTS		alphanumeric, maximum 150 characters

Flounder liver sections and gross liver properties (FL_LIVER)

Description	Field	Required Field	Data type & format
Identifier of sampling event (survey)	EVENT_ID	Y	alphanumeric, maximum 10 characters
Identifier for station.	STAT_ID	Y	alphanumeric, maximum 10 characters
Sample identifier	SAMPLE_ID	Y	alphanumeric, maximum 15 characters
Liver color	LIVER_COL		alphanumeric, maximum 10 characters
Qualifier for LIVER_COL	LIVER_COL_QUAL		alphanumeric, maximum 4 characters
Gross lesions visible on whole flounder liver (0-4)	GROSS_LIV_LESIONS		alphanumeric, maximum 10 characters
Qualifier for GROSS_LIV_LESIONS	GROSS_LIV_LESIONS _QUAL		alphanumeric, maximum 4 characters
Code for lab that performed the analysis.	ANAL_LAB_ID		alphanumeric, maximum 4 characters
Number assigned by the laboratory to the sample.	LAB_SAMPLE_ID		alphanumeric, maximum 35 characters
Comments on the record.	COMMENTS		alphanumeric, maximum 150 characters

Flounder histopathology (FL_LIVHIST)

Description	Field	Required Field	Data type & format
Identifier of sampling event (survey)	EVENT_ID	Y	alphanumeric, maximum 10 characters
Identifier for station.	STAT_ID	Y	alphanumeric, maximum 10 characters
Sample identifier	SAMPLE_ID	Y	alphanumeric, maximum 15 characters
Identifier for sub-sample bottle (generally corresponds to number on label).	BOTTLE_ID	Y	alphanumeric, maximum 15 characters

Description	Field	Required Field	Data type & format
Apoptopic lesion prevalence, rated on a scale from 0-4	BALLOONS		integer
Qualifier for BALLOONS	BALLOONS_QUAL		alphanumeric, maximum 4 characters
Biliary proliferation	BIL_PROLIF		floating point(126 characters
Qualifier for BIL_PROLIF	BIL_PROLIF_QUAL		alphanumeric, maximum 4 characters
Centrotubular hydropic vacuolation	CENTRO_HV		integer
Qualifier for CENTRO_HV	CENTRO_HV_QUAL		alphanumeric, maximum 4 characters
Focal hydropic vacuolation	FOCAL_HV		integer
Qualifier for FOCAL_HV	FOCAL_HV_QUAL		alphanumeric, maximum 4 characters
Macrophage aggregation, rated on a scale from 0-4.	MACROPHAGE		integer
Qualifier for MACROPHAGE	MACROPHAGE _QUAL		alphanumeric, maximum 4 characters
Neoplasia prevalence, rated on a scale from 0-4.	NEOPLASM		integer
Qualifier for NEOPLASM	NEOPLASM_QUAL		alphanumeric, maximum 4 characters
Tubular hydropic vacuolation	TUBULAR_HV		integer
Qualifier for TUBULAR_HV	TUBULAR_HV _QUAL		alphanumeric, maximum 4 characters
Flounder liver flukes	LIVER_FLUKES		integer
Qualifier for LIVER_FLUKES	LIVER_FLUKES _QUAL		alphanumeric, maximum 4 characters
Flounder liver bile duct unicellular parasites	BILE_DUCT_ PROTOZOAN		integer
Qualifier for BILE_DUCT_ PROTOZOAN	BILE_DUCT_ PROTOZOAN_QUAL		alphanumeric, maximum 4 characters
Code for method used for analysis.	METH_CODE		alphanumeric, maximum 13 characters
Code for lab that performed the analysis.	ANAL_LAB_ID		alphanumeric, maximum 4 characters
Number assigned by the laboratory to the sample.	LAB_SAMPLE_ID		alphanumeric, maximum 35 characters
Comments on the record.	COMMENTS		alphanumeric, maximum 150 characters

Lobster morphlogy measurement data (LO_MORPH)

Description	Field	Required Field	Data type & format
Identifier of sampling event (survey)	EVENT_ID	Y	alphanumeric, maximum 10 characters
Identifier for station.	STAT_ID	Y	alphanumeric, maximum 10 characters
Sample identifier	SAMPLE_ID	Y	alphanumeric, maximum 10 characters
Carapace length (mm)	CARAP_LEN	Y	floating point
Qualifier for CARAP_LEN	CARAP_LEN_QUAL		alphanumeric, maximum 3 characters
Wet weight of organism (g)	WEIGHT	Y	floating point
Qualifier for WEIGHT	WEIGHT _QUAL		alphanumeric, maximum 3 characters
Gender	SEX	Y	alphanumeric, maximum 10 characters
Qualifier for SEX	SEX_QUAL		alphanumeric, maximum 3 characters
Shell erosion	SHELL_EROS		alphanumeric, maximum 10 characters
Qualifier for SHELL_EROS	SHELL_EROS_QUAL		alphanumeric, maximum 3 characters
External tumors	EXT_TUMORS		alphanumeric, maximum 10 characters
Qualifier for EXT_TUMORS	EXT_TUMORS_QUAL		alphanumeric, maximum 3 characters
Black gill disease	BLACK_GILL		alphanumeric, maximum 10 characters
Qualifier for BLACK_GILL	BLACK_GILL _QUAL		alphanumeric, maximum 3 characters
Parasite prevalence, rated on a scale from 0-4.	PARASITES		alphanumeric, maximum 10 characters
Qualifier for PARASITES	PARASITES_QUAL		alphanumeric, maximum 3 characters
Code for lab that performed the analysis.	ANAL_LAB_ID		alphanumeric, maximum 4 characters
Number assigned by the laboratory to the sample.	LAB_SAMPLE_ID		alphanumeric, maximum 35 characters
Comments for a given sample (lobster)	SAMPLE_COMMENTS		alphanumeric, maximum 150 characters

Mussel morphology- none

c. Tissue chemistry composites and subsamples

Tissue composites (flounder and lobster) (FL_COMPOSITE, LO_COMPOSITE)
Handle tissue composites in the following way:

1. Each lobster or flounder collected is assigned a SAMPLE_ID and then a BOTTLE_ID representing the whole organism on which morphology and external abnormalities are measured.

Even though the tissues meat are dissected from several lobsters or flounders individually and then composited by tissue type, we treat the processes in the database as if the organisms were composited before the various fractions are removed. A single composite SAMPLEC_ID that represents all the lobsters or flounders in the composite sample. Create a COMPOSITE table with one record for each individual in the composite. In the example below fifteen flounder were composited in groups of five, resulting in three "composite" flounder FF15110C1, FF15110C2, and FF15110C3.

EVENT_ID		SAMPLE_ID	SAMPLEC_ID
FF151		FF1511001	FF15110C1
FF151		FF1511002	FF15110C1
FF151		FF1511003	FF15110C1
FF151		FF1511004	FF15110C1
FF151		FF1511005	FF15110C1
FF151		FF1511006	FF15110C2
FF151		FF1511007	FF15110C2
FF151		FF1511008	FF15110C2
FF151		FF1511009	FF15110C2
FF151		FF1511010	FF15110C2
FF151		FF1511011	FF15110C3
FF151		FF1511012	FF15110C3
FF151		FF1511013	FF15110C3
FF151		FF1511014	FF15110C3
FF151		FF1511015	FF15110C3

2. Assign the SAMPLEC_ID to the composite tissue. The composite sample can now be subsampled, creating new bottles (BOTTLE_ID) for each tissue type (HEPATOPANC and MEAT for lobster, FILLET and LIVER for flounder.)

Description	Field	Required Field	Data type & format
Identifier of sampling event (survey)	EVENT_ID	Y	alphanumeric, maximum 10 characters
Sample identifier	SAMPLE_ID	Y	alphanumeric, maximum 10 characters
Composite sample ID	SAMPLEC_ID	Y	alphanumeric, maximum 10 characters

Tissue chemistry subsample (flounder, lobster) (FL_BOTTLE_CH, LO_BOTTLE_CH)

Description	Field	Required Field	Data type & format
Identifier of sampling event. (survey)	EVENT_ID	Y	alphanumeric, maximum 10 characters
Sample identifier (Corresponds to **SAMPLEC_ID** in the composite table.)	SAMPLE_ID	Y	alphanumeric, maximum 15 characters
Subsample (bottle) identifier (= MWRA DLS sample number)	BOTTLE_ID	Y	alphanumeric, maximum 15 characters
Fraction code (LIVER or FILLET for flounder, MEAT or HEPATOPANC for lobster)	FRACTION_CODE	Y	alphanumeric, maximum 20 characters
Comments for a given bottle	COMMENTS		alphanumeric, maximum 150 characters

Tissue chemistry subsample (mussel) (MY_BOTTLE_CH)

Description	Field	Required Field	Data type & format
Identifier of sampling event. (survey)	EVENT_ID	Y	alphanumeric, maximum 10 characters
Sample identifier	SAMPLE_ID	Y	alphanumeric, maximum 15 characters
Subsample (bottle) identifier (= MWRA DLS sample number)	BOTTLE_ID	Y	alphanumeric, maximum 15 characters
Comments for a given bottle	COMMENTS		alphanumeric, maximum 150 characters

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