

**Quality Assurance
Project Plan**

for

**Fish and Shellfish Monitoring:
2020-2022**

Massachusetts Water Resources Authority
Environmental Quality Department
Report 2020-03



Citation:

Rutecki, D, Nestler, EC, MJ Moore. 2020. **Quality Assurance Project Plan for Fish and Shellfish Monitoring: 2020-2022**. Boston: Massachusetts Water Resources Authority. Report 2020-03. 76 p. plus Appendices A to C.

Environmental Quality Department reports can be downloaded from
<http://www.mwra.com/harbor/enquad/trlist.html>

QUALITY ASSURANCE PROJECT PLAN

for

Fish and Shellfish Monitoring: 2020-2022

MWRA Harbor and Outfall Monitoring Project Contract No. OP401B

Submitted to

Massachusetts Water Resources Authority
Environmental Quality Department
100 First Avenue
Charlestown Navy Yard
Boston, MA 02129
(617) 242-6000

Prepared by

Deborah A. Rutecki¹
Eric C. Nestler¹
Michael J. Moore²

¹Normandeau Associates, Inc.
25 Nashua Road
Bedford, NH 03110

²Woods Hole Oceanographic Institution
Woods Hole, MA 02543

April 2020

Report No. 2020-03

A. PROJECT MANAGEMENT

A1. TITLE AND APPROVALS

**QUALITY ASSURANCE PROJECT PLAN
for
FISH AND SHELLFISH MONITORING: 2020-2022**

**MWRA Harbor and Outfall Monitoring Project
Contract No. OP401B**

Prepared by
**Normandeau Associates, Inc.
and
Woods Hole Oceanographic Institution
April 2020**

Review and Approvals

| | |
|---|---------------------------|
| <u>Signature on file</u> Mr. Eric Nestler Normandeau Project Manager | <u>5/1/2020</u> Date |
| <u>Signature on file</u> Mr. Corey Francis Normandeau Project QA Officer | <u>5/1/2020</u> Date |
| <u>Signature on file</u> Dr. Michael Moore WHOI Principal Investigator (Flounder) | <u>5/1/2020</u> Date |
| <u>Signature on file</u> Mr. Kenneth E. Keay MWRA Project Manager | <u>05/04/2020</u> Date |
| <u>Signature on file</u> Dr. Lucner Charlestra MWRA Fish and Shellfish Project Area Manager | <u>05/04/2020</u> Date |
| <u>Signature on file</u> Dr. Douglas Hersh MWRA EM & MS Manager | <u>05/04/2020</u> Date |

A2. TABLE OF CONTENTS

A. PROJECT MANAGEMENT 1

A1. TITLE AND APPROVALS 1

A2. TABLE OF CONTENTS..... 2

A3. DISTRIBUTION LIST 6

A4. PROJECT AND TASK ORGANIZATION 7

 A4.1 QAPP Introduction..... 7

 A4.2 Project Organization..... 7

 A4.3 Task Organization..... 10

A5. PROBLEM DEFINITION AND BACKGROUND 11

 A5.1 Background 11

 A5.2 Fish and Shellfish Monitoring..... 11

 A5.3 Objectives and Scope 11

A6. PROJECT/TASK DESCRIPTION 13

 A6.1 Project Overview..... 13

 A6.2 Winter Flounder Survey (Task 8)..... 13

 A6.3 Lobster Survey (Task 9)..... 18

 A6.4 Mussel Bioaccumulation Survey (Task 10) 18

 A6.5 Flounder Histology Analysis (Task 11)..... 20

 A6.6 Tissue contaminant analysis preparation (Task 12) 20

 A6.6.1 Flounder (Task 12.1)..... 21

 A6.6.2 Lobster (Task 12.2)..... 21

 A6.6.3 Mussels (Task 12.3)..... 21

 A6.7 Flounder Report (Task 15)..... 22

 A6.8 Schedule of Activities and Deliverables 22

A7. QUALITY OBJECTIVES AND CRITERIA 24

 A7.1 Field Activities 24

 A7.1.1 Navigation..... 24

 A7.1.2 Flounder Survey..... 24

 A7.1.3 Lobster Survey 25

 A7.1.4 Mussel Bioaccumulation Survey 25

 A7.2 Laboratory Activities..... 25

 A7.2.1 Histological Analysis..... 25

 A7.2.2 Flounder Age Analysis 25

 A7.2.3 Tissue Contaminant Analysis Preparation 25

A8. SPECIAL TRAINING AND CERTIFICATIONS 26

 A8.1 Special Training..... 26

 A8.1.1 Field Activities..... 26

 A8.1.2 Laboratory Activities 26

 A8.2 Certifications..... 26

A9. DOCUMENTS AND RECORDS..... 27

 A9.1 Documentation 27

 A9.2 Field Records 27

 A9.3 Laboratory Records 33

 A9.4 Reports and Data Submissions..... 33

 A.9.4.1 QAPP 33

 A.9.4.2 Survey Plan 33

 A.9.4.3 Survey Summary 34

| | | |
|-----------|--|-----------|
| A.9.4.4 | Survey Report..... | 34 |
| A.9.4.5 | Data Reports..... | 35 |
| A.9.4.6 | Annual Flounder Report..... | 35 |
| A.9.5 | Project Files..... | 35 |
| B. | DATA GENERATION AND ACQUISITION | 37 |
| B1. | SAMPLING PROCESS DESIGN (EXPERIMENTAL DESIGN) | 37 |
| B2. | SAMPLING METHODS | 37 |
| B2.1 | Flounder Collection..... | 37 |
| B2.2 | Flounder Shipboard Processing..... | 39 |
| B2.3 | Lobster Collection..... | 40 |
| B2.4 | Mussel Deployment and Collection..... | 42 |
| B2.5 | Flounder Processing for Tissue Contaminant Analysis..... | 44 |
| B2.6 | Lobster Processing for Tissue Contaminant Analysis..... | 44 |
| B2.7 | Mussel Processing for Tissue Contaminant Analysis..... | 45 |
| B3. | SAMPLE HANDLING AND CUSTODY | 46 |
| B3.1 | Sample Storage..... | 46 |
| B3.2 | Sample Identification | 46 |
| B3.3 | Sample Custody..... | 46 |
| B3.4 | Sample Archival Policy..... | 47 |
| B4. | ANALYTICAL METHODS | 48 |
| B4.1 | Histological analysis..... | 48 |
| B4.2 | Age Determination | 48 |
| B5. | QUALITY CONTROL..... | 49 |
| B5.1 | Sampling..... | 49 |
| B5.1.1 | Navigation..... | 49 |
| B5.1.2 | Flounder Survey..... | 49 |
| B5.1.3 | Lobster Survey | 51 |
| B5.1.4 | Mussel Bioaccumulation Survey | 51 |
| B5.2 | Flounder Histological Analysis and Age Determination | 52 |
| B6. | INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE..... | 54 |
| B6.1 | Field Equipment Maintenance Requirements | 54 |
| B6.2 | Laboratory Equipment | 54 |
| B7. | INSTRUMENT/EQUIPMENT CALIBRATION AND FREQUENCY | 55 |
| B7.1 | Navigation Equipment..... | 55 |
| B7.2 | Laboratory Equipment | 55 |
| B8. | INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES | 56 |
| B9. | NONDIRECT MEASUREMENTS | 56 |
| B10. | DATA MANAGEMENT | 57 |
| B10.1 | Data Custody..... | 57 |
| B10.2 | Data Entry and Processing | 57 |
| B10.3 | Data Reduction..... | 58 |
| B10.4 | Data Set Structure | 58 |
| B10.5 | Project Database Codes..... | 58 |
| B10.6 | Data Submittal to MWRA..... | 58 |
| B10.7 | Data Report Quality Control Checks..... | 59 |
| C. | ASSESSMENT AND OVERSIGHT..... | 63 |
| C1. | ASSESSMENT AND RESPONSE ACTIONS | 63 |
| C1.1 | Planned Assessments..... | 63 |
| C1.2 | Assessment Findings and Corrective Action Responses | 63 |

C2. REPORTS TO MANAGEMENT 69

D. DATA VALIDATION AND USABILITY 70

D1. DATA REVIEW, VERIFICATION, AND VALIDATION..... 70

 D1.1 Field Data 70

 D1.2 Laboratory Data..... 70

 D1.3 Data Management..... 70

D2. VALIDATION AND VERIFICATION METHODS 71

 D2.1 Field Data 71

 D2.2 Laboratory Data..... 71

 D2.3 Data Management..... 71

 D2.4 Project Deliverables..... 72

D3. RECONCILIATION WITH USER REQUIREMENTS 73

 D3.1 Comparison to Measurement Criteria 73

 D3.1.1 Precision and Accuracy Assessment..... 73

 D3.1.2 Completeness Assessment 73

 D3.1.3 Representativeness..... 73

 D3.2 Overall Assessment of Environmental Data..... 74

E. REFERENCES 75

LIST OF FIGURES

Figure 1. Flounder monitoring locations for 2020, 2021, and 2022. 14

Figure 2. Lobster monitoring locations for 2021. 15

Figure 3. Mussel collection and deployment locations for 2021. 16

Figure 4. Chain-of-custody form for flounder, lobster, and mussel tissue contaminant analysis samples. 28

Figure 5. Field log sheet for flounder sampling (Station log sheet). 29

Figure 6. Sample log sheet for recording flounder data in the field. 30

Figure 7. Lobster survey field log..... 31

Figure 8. Mussel survey field log. 32

Figure 9. Length measurements for winter flounder. 39

Figure 10. Carapace measurement of lobster..... 41

Figure 11. Length Measurement of Mussels..... 42

LIST OF TABLES

Table 1. Personnel Responsibilities and Contact Information..... 9

Table 2. Sampling Locations for Flounder, Lobster, and Mussel Surveys..... 17

Table 3. Minimum number of mussels required for each location..... 19

Table 4. Schedule of Fish and Shellfish Monitoring Deliverables..... 22

Table 5. Monitoring Parameters, Collection Frequency, Sample Containers, Preservation, and Holding Time Requirements 38

Table 6. Precision and Accuracy of Navigation Data. 49

Table 7. Data Quality Objectives (DQOs) for Physical Measurements of Flounder and Lobster 50

Table 8. Supplies, Acceptance Criteria, and Responsibility for Critical Field Supplies. 56

Table 9. Data Qualifiers 59

Table 10. Morphological Parameters and Database Codes for Fish and Shellfish Monitoring..... 59

Table 11. Histopathological Parameters and Database Codes for Fish and Shellfish Monitoring. 59

Table 12. General Database Codes for Fish and Shellfish Monitoring. 60

Table 13. Range Checks for Flounder and Lobster Monitoring..... 61
Table 14. Planned Project Assessments 65
Table 15. Example of Field Audit Checklist 67
Table 16. Example of Laboratory Audit Checklist..... 68

APPENDICES

- Appendix A. Guidance for Recording External Lesions in Flounder
- Appendix B. Flounder Tissue Resection SOP
- Appendix C. Specifications for Data Sets

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 | |
| Eric Nestler, Normandeau | |
| Corey Francis, Normandeau | |
| Deborah Rutecki, Normandeau | |
| Erik Fel'Dotto, Normandeau | |
| Vivian English, Experimental Pathology Laboratories | |
| Kirk Cram, Enthalpy | |

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 (HOM11 Contract OP401). 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 (EPA 2001, EPA 2002, Maciolek et al. 2010, Lao et al. 2012, Nestler et al. 2013, Geoghegan et al. 2016, Rutecki et al. 2017). 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.

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.
- Dr. Douglas Hersh, 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

- Mr. Eric Nestler, Normandeau Project Manager, is responsible for the overall performance of this project and for ensuring that products and services meet MWRA's expectations, and are delivered in a timely and cost-effective manner. He is responsible for ensuring that data collection and interpretation are scientifically defensible, and for responding to technical challenges as they arise.
- Ms. Ann Pembroke, Normandeau Corporate Officer and Technical Advisor, 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. She will also provide the overall technical review for the project.
- Mr. Corey Francis, 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. Francis will also review and approve the health and safety plans and procedures for the project.

- Ms. Deborah Rutecki, Normandeau's Deputy Project Manager, will prepare the QAPP and assist in overall project management including the lobster survey and mussel bioaccumulation monitoring.
- Mr. Erik Fel'Dotto, Normandeau's Field Manager for the mussel survey, will oversee the field aspects of the mussel bioaccumulation survey.
- Dr. Chris Gurshin is Normandeau's Statistical Advisor for the project and will assist as needed with data analysis and interpretation.
- Mr. Paul Geoghegan will provide overall technical review for the project.

WHOI

- Dr. Michael Moore is the Principal Investigator for the Flounder Monitoring Task. He is 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. 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. He will prepare the annual reports on the results of the flounder tissue analysis.

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. Kirk Cram, Enthalpy Analytical (Enthalpy), 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 2021.

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 | | | |
| Mr. Eric Nestler | Normandeau Associates, Inc. 25 Nashua Road Bedford, NH 03110 | Program Manager (All Tasks) | Ph: (603) 637-1146 Fax: (603) 472-7052 enestler[at]normandeau.com |
| Ms. Deborah Rutecki | Normandeau Associates, Inc. 141 Falmouth Heights Road Falmouth, MA 02540 | Deputy Project Manager (All Tasks) | Ph: (508) 815-5505 Fax: (508) 548-2004 drutecki[at]normandeau.com |
| Ms. Ann Pembroke | Normandeau Associates, Inc. 25 Nashua Road Bedford, NH 03110 | Corporate Officer and Technical Advisor (All Tasks) | Ph: (603) 637-1169 Fax: (603) 472-7052 apembroke[at]normandeau.com |
| Mr. Paul Geoghegan | | Technical Advisor (Tasks 3, 9 and 10) | Ph: (603) 637-1163 Fax: (603) 472-7052 pgeoghegan[at]normandeau.com |
| Mr. Corey Francis | | Project QA Officer (Tasks 3 and 4); Project Health and Safety Officer | Ph: (603) 637-1173 Fax: (603) 472-7052 cfrancis[at]normandeau.com |
| Mr. Erik Fel'Dotto | Normandeau Associates, Inc. 43 Harbor Road Hampton, NH 03842 | Field Manager - Mussel Survey; (Task 10) | Ph: (603) 319-5072 Cell: (603) 345-9813 efeldotto[at]normandeau.com |
| Dr. Chris Gurshin | Normandeau Associates, Inc. 30 International Drive, Suite 6 Portsmouth, NH 03801 | Project Statistician (Tasks 13 and 15) | Ph: (603) 319-5304 Fax: (603) 334-6397 cgurshin[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. Kirk Cram | Enthalpy Analytical P.O. Box 778 One Lafayette Road Hampton, NH 03842 | Tissue contaminant analysis preparation (Task 12) | Ph: (603) 926-3345 ext. 15003 Ph: (603) 651-7903 (cell) ksimon[at]Enthalpy.com |
| Mr. Mark Carroll | Not available at this time. | Captain, F/V <i>Harvest Moon</i> (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 OP401 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 2021 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 2021). 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, Constantino J, Leo W, Delaney MF, Epelman P, Rhode S. 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 p.

Moore MJ, Nestler EC, Rutecki DA. 2018. Flounder monitoring report: 2018 results. Boston: Massachusetts Water Resources Authority. Report 2018-08. 18 p.

Moore MJ, Nestler EC, Rutecki DA. 2019. 2019 Flounder monitoring results. Boston: Massachusetts Water Resources Authority. Report 2019-10. 21 p.

Moore MJ, McElroy AE, Geoghegan P, Siskey MR, Pembroke AE. 2016. Flounder Monitoring Report: 2015 Results. Boston: Massachusetts Water Resources Authority. Report 2016-05. 40 p.

Moore MJ, Pembroke A, Nestler EC, Hall M, Lefkovitz L, Lambert M, Keay K. 2018. Toxics source reduction and sewage upgrades eliminated winter flounder liver neoplasia (1984-2017) from Boston Harbor, MA, USA. *Diseases of Aquatic Organisms* 131:239-243.

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 2020 and 2022, only winter flounder will be collected, and chemical analyses will not be run. Flounder, lobster, and mussel surveys will all be conducted during 2021, 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 (2020, 2021 and 2022) – Task 8
2. Lobster Survey (2021) – Task 9
3. Mussel Bioaccumulation Survey (2021) – Task 10
4. Flounder Histology Analysis (2020, 2021 and 2022) – Task 11
5. Tissue Contaminant Analysis Preparation (2021) – 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 2020, 2021 and 2022 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. Scales (2020-2022) will be removed from each flounder for age determination in the laboratory.



Figure 1. Flounder monitoring locations for 2020, 2021, and 2022.

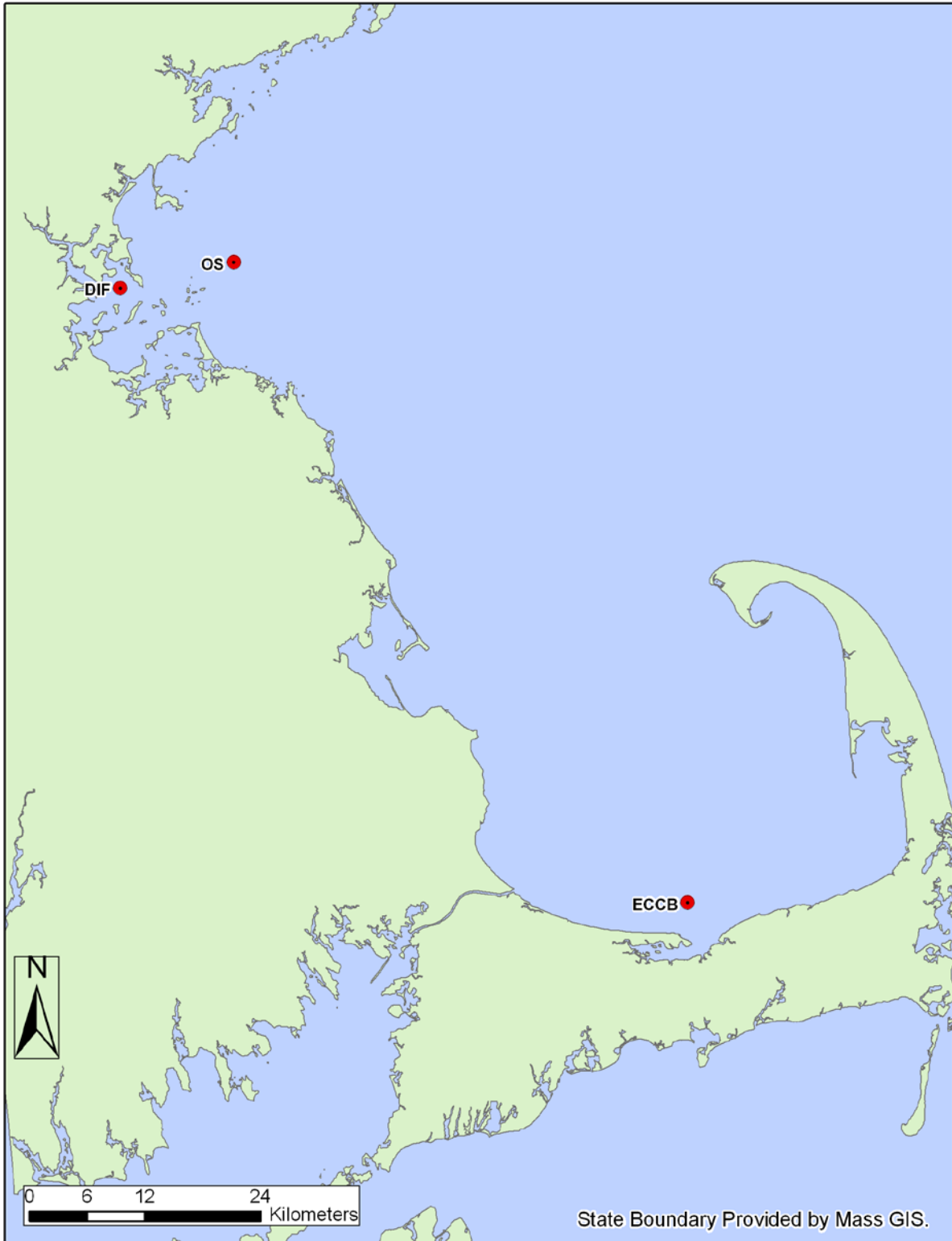


Figure 2. Lobster monitoring locations for 2021.

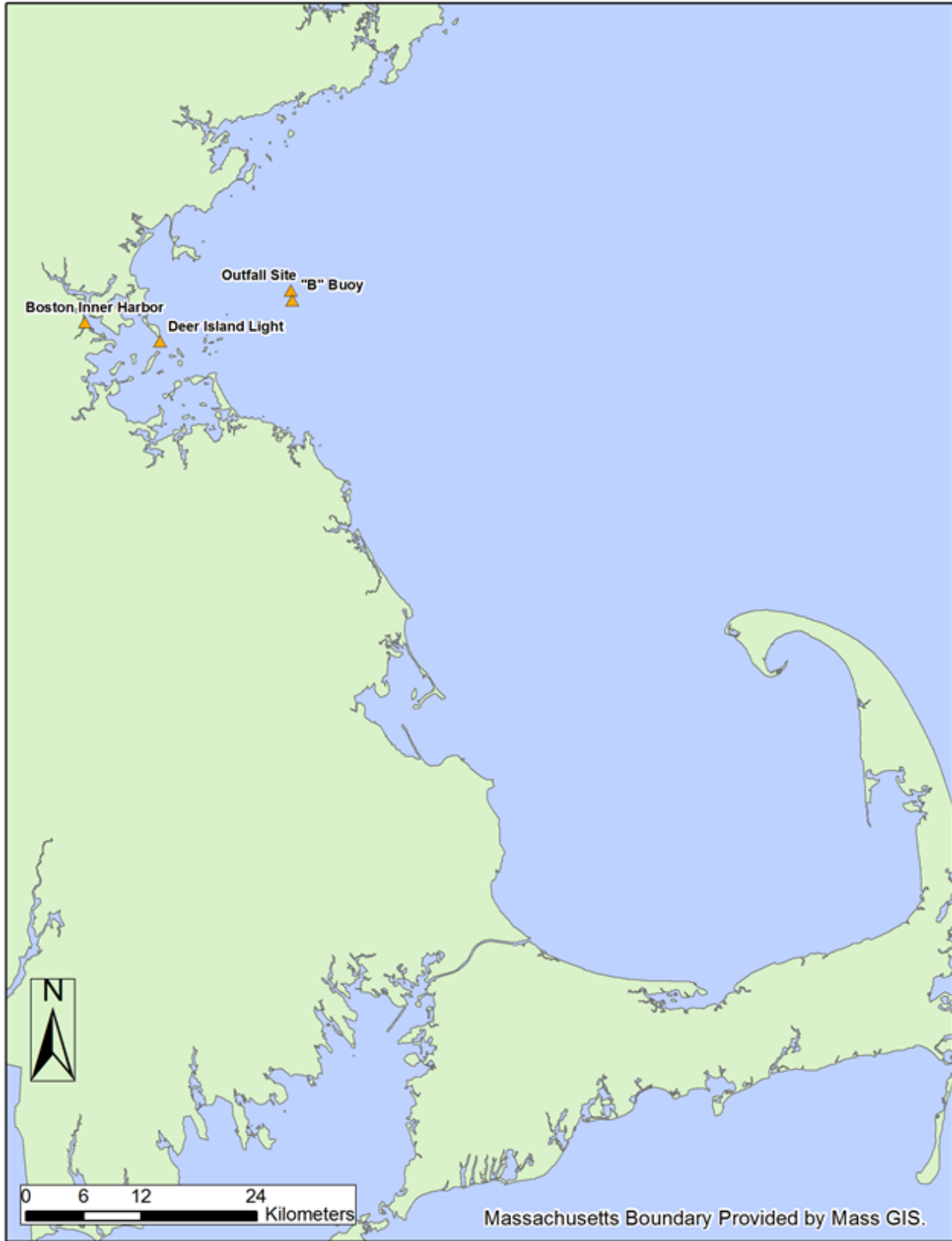


Figure 3. Mussel deployment locations for 2021.

Table 2. Sampling 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' | | | * |
| To Be Determined ⁺ | To Be Determined | Pre-survey Condition (To Be Determined) | | | | | 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”. + The mussel collection location will be determined following the site selection process described in Section B2.4.

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 2021, 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 Enthelphy in Hampton, NH. Liver and edible tissue (skin-free fillet) will be aseptically removed by Enthelphy (Task 8.6). 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 <https://www.fisheries.noaa.gov/topic/marine-life-viewing-guidelines>). 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 2021. 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 2021 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 differential Global Positioning System (dGPS) 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 Enthalpy where tissue samples will be obtained in a clean room environment. Total lobster weight measurements (in grams) and black gill disease assessments will be conducted in the lab by Enthalpy.

A6.4 Mussel Bioaccumulation Survey (Task 10)

A mussel bioaccumulation survey will be conducted between late June and late August 2021 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.

Intertidal mussel populations have declined at the sites where specimens have been collected in the past for this survey and throughout the Gulf of Maine. Additionally, paralytic shellfish poisoning (PSP) closures can interfere with access to preferred mussel beds. During the 2015 and 2018 surveys, insufficient numbers of wild mussels from Maine intertidal populations, along with widespread and prolonged PSP closure periods prohibited the collection of wild mussels. At the recommendation of Maine Department of Marine Resources and with MWRA's approval, farmed mussels were used for the studies in these years. If wild mussel populations are available, test mussels will be collected from a clean reference location in Maine. Normandeau will obtain a special license from Maine Department of Marine Resources, if required, so that sufficient mussels for the pre-survey and post-survey analyses can be collected. If wild intertidal mussels are insufficient, Normandeau will contact Acadia Aqua Farms,

which sourced mussels in 2018, and other aquaculture operations in order to obtain sufficient mussels for the pre-survey and post-survey analyses (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. Previous bioaccumulation surveys, using wild mussels, 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. Unlike wild mussels collected intertidally, farmed mussels can exhibit extremely high mortality (as much as 83% at IH and as high as 65% offshore in 2015, and 37% at IH in 2018; Pembroke et al. 2015, Normandeau 2018) in the deployment cages. Should farmed mussels be required, Normandeau will increase the number deployed at each station accordingly, including adding additional arrays to avoid overcrowding if necessary.

Mussels will be measured to ensure that the average length is approximately 6 cm. In the event that the supply of wild intertidal mussels is insufficient and farmed mussels need to be used, the number of mussels deployed at each station will be increased including adding additional arrays to avoid overcrowding if necessary.

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% |
| Pre-survey Condition (OP) | 4 | 100 |
| Total | 44 + 12 | 1400 + 15% |

All of the potential sources of mussels identified to date are in areas where there are fewer discharges than Massachusetts Bay. However, many contaminants can be transported from great distances atmospherically. Knowledge of baseline levels of contaminants in mussels (Task 10.3.1) 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 10.3.2) 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 2021 has been completed by MWRA. Thus, approximately 1,600 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 10.4). 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 2021, 60 days after deployment, mussels will be retrieved from the remaining arrays (Task 10.5). 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 scale analysis by Normandeau Associates in 2020, 2021, and 2022. 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 2020, 2021, and 2022. 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 \times , 100 \times , and 200 \times , 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) 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 2021 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)

Enthalpy, Inc., a toxicity and analytical laboratory located in Hampton NH will composite the tissue samples collected during 2021 prior to delivery to MWRA's DLS. Enthalpy 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 2021, 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 and homogenized to form one composite sample. Each composite will be placed in a clean sample container, labeled, and frozen.

Filletts will be removed from the flounder and the skin will be removed from the filletts 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 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 2021, 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 2021, 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 2, 2020) until completion of the final report in 2022. 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 2020-2022 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 2020 Final: April 2020 |
| Flounder Survey (Task 8) | Survey Plan Survey Cruise Survey Summary E-mail Survey Report | March 2020, 2021, 2022 April 2020, 2021, 2022 2 days after survey May 2020, 2021, 2022 |
| Lobster Survey (Task 9) | Survey Plan Survey Cruise Survey Summary E-mail Survey Report | June 2021 July 2021 2 days after survey September 2021 |
| Mussel Bioaccumulation Survey (Task 10) | Survey Planning Letter Report Survey Plan Mussel Deployment Mussel 40-day Collection Mussel 60-day Collection Survey Summary E-mail Survey Report | October 1, 2020 May 2021 June 2021 Early August 2021 Late August 2021 2 days after survey September 2021 |
| Flounder Survey Data (Task 4) | Survey Data Tissue Composite Data | May 15, 2020, 2021, 2022 May 15, 2021 |
| Lobster Survey Data (Task 4) | Survey Data Tissue Composite Data | Both due 15 days after survey completion in 2021 |

| | | |
|---|--------------------------------------|---|
| Mussel Bioaccumulation Survey Data (Task 4) | Survey Data Tissue Composite Data | September 15,2021 September 15, 2021 |
| Flounder Histology Analysis (Tasks 4 and 11) | Histology Data | August 15, 2020, 2021, 2022 (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 2020, 2021, 2022 |
| Annual Technical Meeting (Task 14) | Presentation | March 2021, 2022, 2020 |
| Annual Flounder Report (Task 15) | Draft Report Final Report | October 2020, 2021, 2022 November 2020, 2021, 2022 |

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 (Rutecki et al. 2017).

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, 3,000 m; ECCB, 10,000 m; NB, 2,000 m; and OS, 3,000 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 Wide Area Augmentation System (WAAS) enabled GPS navigation system will be used to acquire navigation data for mussel surveys. The deployment of mussels will be conducted within 3 m of the targets as determined by Normandeau's WAAS enabled GPS.

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 1,600) 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 mussel 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 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 scales according to the methods of Fields (1988).

In 2021, Enthalpy 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 Enthalpy 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 HOM11 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 HOM11 project files and to Dr. Charlestra (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: <input style="width: 100%;" type="text"/> Project Number: <input style="width: 100%;" type="text"/> Originating Contact: <input style="width: 100%;" type="text"/> Originator Location: <input style="width: 100%;" type="text"/> Final Destination: <input style="width: 100%;" type="text"/> Sampler(s): <input style="width: 100%;" type="text"/> | | | | | Parameters | | | | Preservative | | | | Comments |
|---|---------|--------------------------|------------|------------------------------|------------|--------------------------|--|--------------------|--------------|---------------------|--|--|----------|
| | | | | | | | | | | | | | |
| | | | | | | | | | Frozen | | | | |
| Sample No.* | Station | Replicate | Collection | | | | | | | | | | |
| | | | Type | Date | Time | | | | | | | | |
| | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| | | | | Total | | | | | | | | | |
| Relinquished by: (signature) | | Received by: (signature) | | Relinquished by: (signature) | | Received 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
 DGPS or GPS or LORAN
 ±10m and ±30m

VESSEL:
 NAVIGATION_CODE:
 NAV_QUAL:
 VESSEL CREW:
 SCIENCE CREW:

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

| ID | Station Name | Weight. (g) | TL mm | SL mm | Sex | Age | Fin Rot (0-4) | Bent Fin Ray (0-4) | Ulcer (0-4) | Net (0-4) | Lympho. (0-4) | Liver Color* | Liver Gross + | Photo? |
|-------|--------------|-------------|-------|-------|-----|-----|---------------|--------------------|-------------|-----------|---------------|--------------|---------------|--------|
| FF201 | 1016 | Deer Island | | | | | | | | | | | | |
| FF201 | 1017 | Deer Island | | | | | | | | | | | | |
| FF201 | 1018 | Deer Island | | | | | | | | | | | | |
| FF201 | 1019 | Deer Island | | | | | | | | | | | | |
| FF201 | 1020 | Deer Island | | | | | | | | | | | | |
| FF201 | 1021 | Deer Island | | | | | | | | | | | | |
| FF201 | 1022 | Deer Island | | | | | | | | | | | | |
| FF201 | 1023 | Deer Island | | | | | | | | | | | | |
| FF201 | 1024 | Deer Island | | | | | | | | | | | | |
| FF201 | 1025 | Deer Island | | | | | | | | | | | | |
| FF201 | 1026 | Deer Island | | | | | | | | | | | | |
| FF201 | 1027 | Deer Island | | | | | | | | | | | | |
| FF201 | 1028 | Deer Island | | | | | | | | | | | | |
| FF201 | 1029 | Deer Island | | | | | | | | | | | | |
| FF201 | 1030 | Deer Island | | | | | | | | | | | | |
| FF201 | 1031 | Deer Island | | | | | | | | | | | | |
| FF201 | 1032 | Deer Island | | | | | | | | | | | | |
| FF201 | 1033 | 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): _____

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

| SAMPLE ID | CARAPACE LENGTH (mm) | WEIGHT (g) | SEX (F/M) | SHELL EROSION (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 % | 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: _____

Figure 8. Mussel survey field log.

Mussel.ai 6/06

A9.3 Laboratory Records

There are three sources of laboratory records for this project: (1) in 2020, 2021, and 2022 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; (2) histology data read by Dr. Moore from the slides and recorded electronically into Excel, and, (3) internal condition data collected by Enthalpy for flounders (e.g. liver color) and lobsters (e.g. black gill disease) selected for tissue contaminant analyses during 2021.

Dr. Moore will enter the histology data (items 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 2021 will be delivered to the Enthalpy laboratory along with the specimens. Data that could not be collected in the field (e.g. liver color for flounder, or black gill disease and weight 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, Dr. Lucner Charlestra, 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 HOM11 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 2020, 2021, and 2022. 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 <https://www.fisheries.noaa.gov/topic/marine-life-viewing-guidelines>). 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 (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 | Biometrics weight | 21/21 | N/A | Laboratory: Process immediately. Describe quantitatively | NA |
| | 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 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 Reference site ^d : 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.

d = The location of the reference site (i.e. mussel collection location) is to be determined based on mussel availability and following the site selection process described in Section B2.4.

B.2.2 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.

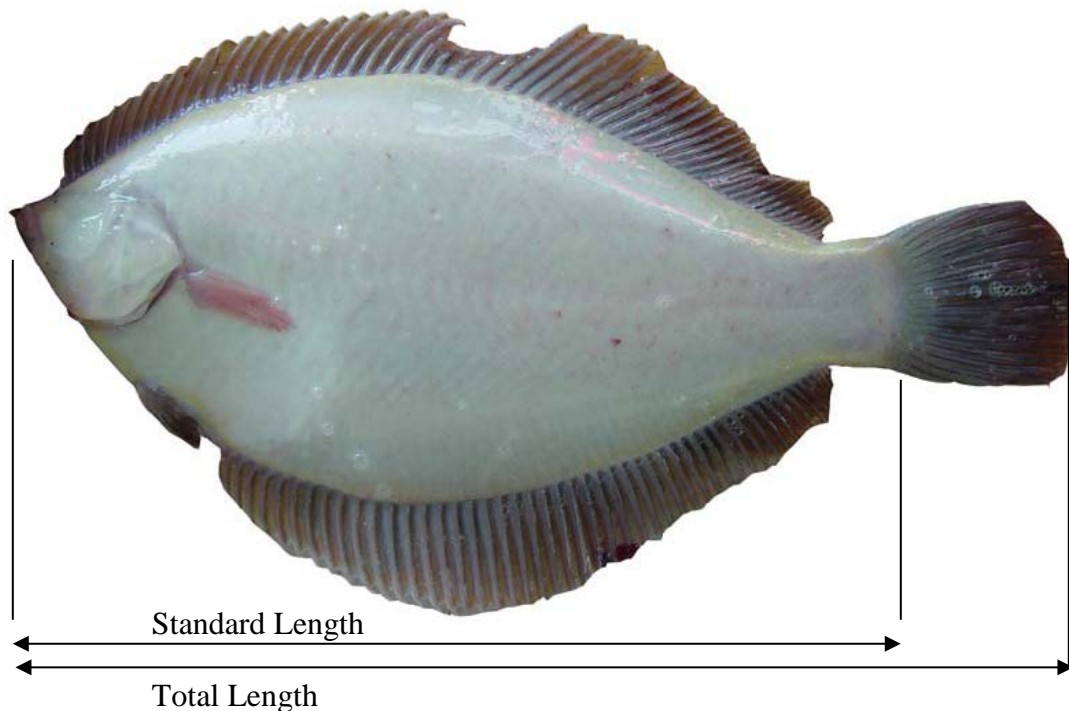


Figure 9. Length measurements for winter flounder.

For 2020 through 2022, scales will also be removed from each flounder following protocols described in Appendix B. Scales will be collected from each specimen for age determination through the analysis of growth rings (annuli) on the scales. A blunt-edged table knife will be used to remove any mucus, debris, or epidermis from the dorsum of the caudal peduncle by wiping in the direction of the tail. Scales will be removed from the cleaned area by applying quick, firm scraping motions in the direction of the head. Scales will be immediately placed in the pre-labeled age-sample envelope by inserting the knife between the liner of the sample envelope and scraping the scales into the envelope. A temporary check mark will be made on the field data sheet to indicate that this sample has been collected. Scale samples will be subsequently sent to Normandeau's Biological Laboratory, in Bedford, NH for age determination. The age data results will be entered on the datasheets, initialed, and dated. These results will be entered into the Excel file together with other results on the same data sheet.

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 2020, 2021, and 2022 will be processed for histopathology. In 2021 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 Enthalpy to be processed in the laboratory under clean, contaminant-free conditions. Normandeau will arrange with Enthalpy for an after-hours delivery if necessary; or a courier from Enthalpy will pick up the fish from the boat.

B2.3 Lobster Collection

Three sites will be sampled to collect lobster for chemical analyses during 2021: (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.

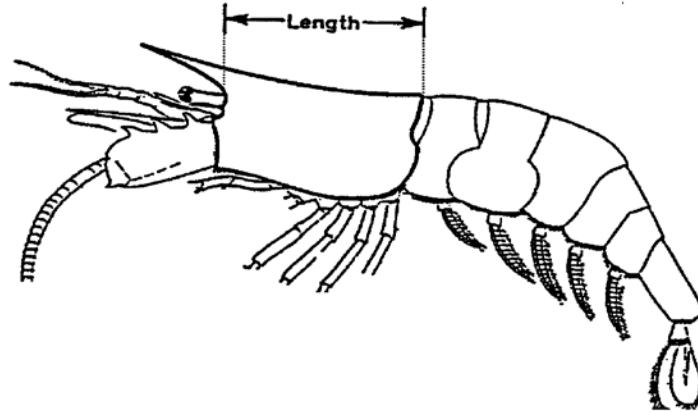


Figure 10. Carapace measurement of lobster

4. The fisheries technician will process the lobsters for physical characteristics (carapace length, gender, and gross external abnormalities, such as shell erosion, parasites, and external tumors); which will 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 Enthalpy to be processed in the laboratory under clean, contaminant-free conditions. Normandeau will arrange with Enthalpy for an after-hours delivery if necessary; or a courier from Enthalpy will pick up the samples from the boat.
8. Total lobster weight measurements (in grams) and black gill disease assessments will be conducted in the lab by Enthalpy. 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 2021, live blue mussels (*Mytilus edulis*) approximately 6 cm in length will be collected from a baseline (pre-survey) reference site or an aquaculture facility located in Maine or Massachusetts. Normandeau will update its license from the Maine Department of Marine Resources for the procurement of these mussels (if required; note that the quantities of mussels required for the project in recent previous years has fallen under the Maine “personal use” harvest limit). 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. Previous bioaccumulation surveys, using wild mussels, 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. Unlike wild mussels collected intertidally, farmed mussels can exhibit extremely high mortality (as much as 83% at IH and as high as 65% offshore in 2015, and 37% at IH in 2018; Pembroke et al. 2015, Normandeau 2018) in the deployment cages. Should farmed mussels be required, the number deployed will be increased at each station accordingly. Additional arrays will be added to avoid overcrowding if necessary. Mussels collected for the bioaccumulation survey will measure approximately 6 cm (55-65mm; Figure 11).

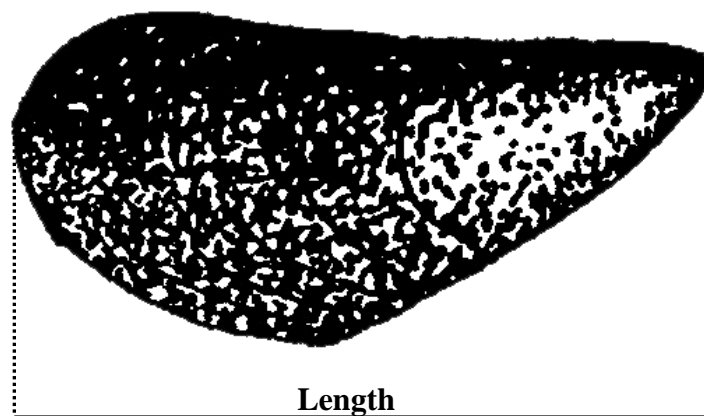


Figure 11. Length Measurement of Mussels.

All of the potential sources of mussels identified to date are in areas where there are 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.

Intertidal mussel populations have declined at the sites where specimens have been collected in the past for this survey and throughout the Gulf of Maine. Additionally, paralytic shellfish poisoning (PSP) closures can interfere with access to preferred mussel beds. During the 2015 and 2018 surveys, insufficient numbers of wild mussels from Maine intertidal populations, along with widespread and prolonged PSP closure periods prohibited the collection of wild mussels. At the recommendation of Maine Department of Marine Resources (DMR) and with MWRA's approval, farmed mussels were used for the studies in these years.

To ensure successful mussel collections in 2021, early communication with the Maine DMR will begin in the summer of 2020 to discuss the recent difficulties in locating healthy wild intertidal mussel populations and to determine if field collection of mussels for scientific purposes is likely to be feasible for the 2021 study. Reconnaissance visits will be made to mussel beds identified by Maine DMR as potential sources of wild mussels. Surveys will be conducted during low tide to determine if mussel populations are sufficient for collection. Consultation will also occur with local shellfish wardens to determine if they have any concerns about our harvest. In the event that the supply of wild intertidal mussels is insufficient, we will contact Acadia Aqua Farms, which sourced mussels in 2018, and other aquaculture operations in coastal Maine. Another alternative to using wild intertidal mussels may also be a pilot offshore mussel aquaculture operation located in Massachusetts waters. We will discuss these options with the likely logistics and constraints with MWRA should the supply of intertidal mussels be insufficient. The results of these planning activities, including recommendations on where to collect mussels, will be presented in a summary report and discussed with 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 2021 has been completed by MWRA. Thus, approximately 1,600 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 WAAS enabled GPS. 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 2021, 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 Enthalpy laboratory. Normandeau will arrange with Enthalpy for an after-hours delivery if necessary; or a courier from Enthalpy 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 Enthalpy, 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 Enthalpy 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 ($\pm 10\%$ 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 ($< 5\text{g}$ 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 Enthalpy 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 Enthalpy 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 ($\pm 10\%$ 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

Enthalpy 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 “FF211”, with “FF” indicating that it is a flounder survey (“FL” for lobster survey and “FM” for mussel survey), “21” 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 Experimental Pathology Laboratories (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 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.

Enthalpy will maintain custody of flounder, lobster and mussel samples for tissue contaminant analysis preparation. The Enthalpy 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, 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 2021 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 Normandeau will be archived at the Normandeau facility 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 \times , 100 \times , and 200 \times 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 otoliths, following procedures described by Fields (1997), with missing data filled in using scales. In 2016, aging was again conducted using scales because of NMFS' limited availability. Age determination in 2020, 2021, and 2022 will be conducted using scales and will be consistent with the methods followed historically for this program (Fields 1988). 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. All WAAS enabled GPS units have a design positional accuracy of 3 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) |
| WAAS enabled GPS Navigation | degree | Coastal* | 9x10 ⁻⁵ deg (<3 m) | 1.8x10 ⁻⁵ deg (<3 m) |

* Available only in North American.

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 or WAAS enabled GPS 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 3 m of the targets as determined by Normandeau’s WAAS enabled GPS.

Completeness

For all navigation data, 100% completeness has been defined as the QAPP requirement. Normandeau’s WAAS enabled GPS 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. DQOs are the

qualitative and quantitative statements derived from the study’s systematic planning process that clarify the technical and quality objectives, define the appropriate type of data, and specify tolerable levels of potential decision errors that will be used as the basis for establishing the quality and quantity of data (EPA 2002).

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

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

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.

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 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 scales will be read by 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 Normandeau 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 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 microfiche used by Normandeau's Biological Laboratory for the age analysis task.

Under HOM11, 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 GPS Map 78SC 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 HOM11, there are no analytical laboratory instruments covered by this QAPP.

B8. INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES

For HOM11, 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 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 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, Normandeau, and Enthalpy 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. The Enthalpy laboratory manager will be responsible for data collected during the processing of flounder, lobster, or mussels. The data from Enthalpy will be transferred on paper datasheets 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 through 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 9 through 12.

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 be documented in the data submission letters, QA statements, and in the comments field in the data files (if applicable) when 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? | Explanatory Comment Required in SAMPLE Data? |
|-----------|---|-----------------|--|
| e | Results not reported, value given is NULL, see comments field | No | Yes |
| w | This datum should be used with caution, see comment field | Yes | Yes |
| s | Suspect/Invalid. Not fit for use | Yes | Yes |
| j | Estimated value | Yes | No |

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

| SPECIES | PARAM_CODE | DESCR | UNIT_CODE | METH_CODE |
|--------------------------------------|------------|---|-----------|-----------|
| <i>Homarus americanus</i> | CARAP_LEN | Carapace Length | mm | CLM |
| <i>Homarus americanus</i> | SEX | Gender | | VISUAL |
| <i>Homarus americanus</i> | WEIGHT | Wet Weight of Organism | g | LWEIGHT |
| <i>Pseudopleuronectes americanus</i> | AGE | Chronological age of specimen | y | SCALE |
| <i>Pseudopleuronectes americanus</i> | SEX | Gender | | VISUAL |
| <i>Pseudopleuronectes americanus</i> | STAN_LEN | Standard length of a fish. From upper jaw tip to posterior end of the hypural bone. | mm | SLM |
| <i>Pseudopleuronectes americanus</i> | TOTAL_LEN | Total Length | mm | TLM |
| <i>Pseudopleuronectes americanus</i> | 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 | <i>Pseudopleuronectes americanus</i> | LIVER_SECTION | BILE_DUCT_PROTOZOAN | Presence (1) or absence (0) of flounder liver bile duct unicellular parasites in histology sections |

| | | | | |
|------------|--------------------------------------|---------------|-------------------|---|
| 8857041504 | <i>Pseudopleuronectes americanus</i> | LIVER_SECTION | BALLOONS | Apoptotic lesion prevalence, rated on a scale from 0-4. |
| 8857041504 | <i>Pseudopleuronectes americanus</i> | LIVER_SECTION | BIL_PROLIF | Biliary proliferation |
| 8857041504 | <i>Pseudopleuronectes americanus</i> | LIVER_SECTION | CENTRO_HV | Centrotubular hydropic vacuolation |
| 8857041504 | <i>Pseudopleuronectes americanus</i> | LIVER_SECTION | FOCAL_HV | Focal hydropic vacuolation |
| 8857041504 | <i>Pseudopleuronectes americanus</i> | LIVER_SECTION | LIVER_FLUKES | Coded score (0-4) for presence of flounder liver flukes in histology sections |
| 8857041504 | <i>Pseudopleuronectes americanus</i> | LIVER_SECTION | MACROPHAGE | Macrophage aggregation, rated on a scale from 0-4. |
| 8857041504 | <i>Pseudopleuronectes americanus</i> | LIVER_SECTION | NEOPLASM | Neoplasia prevalence, rated on a scale from 0-4. |
| 8857041504 | <i>Pseudopleuronectes americanus</i> | LIVER_SECTION | TUBULAR_HV | Tubular hydropic vacuolation |
| 8857041504 | <i>Pseudopleuronectes americanus</i> | WHOLE_BODY | FIN_ROT | Fin rot score |
| 8857041504 | <i>Pseudopleuronectes americanus</i> | WHOLE_BODY | GROSS_LIV_LESIONS | Gross lesions visible on whole flounder liver |
| 8857041504 | <i>Pseudopleuronectes americanus</i> | WHOLE_BODY | ULCER | Flounder skin ulcer |
| 8857041504 | <i>Pseudopleuronectes americanus</i> | WHOLE_BODY | LYMPHO | Lymphocystis |
| 8857041504 | <i>Pseudopleuronectes americanus</i> | WHOLE_BODY | BENT_FIN | Bent fin ray |
| 8857041504 | <i>Pseudopleuronectes americanus</i> | WHOLE_BODY | NET_DAMAGE | Net damage |
| 8857041504 | <i>Pseudopleuronectes americanus</i> | WHOLE_BODY | LIVER_COL | Liver color |
| 6181010201 | <i>Homarus americanus</i> | WHOLE_BODY | BLACK_GILL | Black gill disease |
| 6181010201 | <i>Homarus americanus</i> | WHOLE_BODY | EXT_TUMORS | External tumors |
| 6181010201 | <i>Homarus americanus</i> | WHOLE_BODY | PARASITES | Parasite prevalence, rated on a scale from 0-4. |
| 6181010201 | <i>Homarus americanus</i> | 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 from F/V Odessa, sweep 70 feet, rope length 50 feet, or unspecified otter trawl |
| GEAR_CODE | OTT_EX | Otter trawl tow from F/V Explorer 2, sweep 84 feet, rope length 62 feet |
| GEAR_CODE | OTT_HM | Otter trawl tow from F/V Harvest Moon, sweep 74 feet, rope length 52 feet |
| GEAR_CODE | TRAP | Lobster trap |
| INSTR_CODE | BAL | Balance |
| INSTR_CODE | MICR | Microscope |
| INSTR_CODE | RULER | Measurement by ruler |
| MATRIX_CODE | 5507010101 | <i>Mytilus edulis</i> |
| MATRIX_CODE | 5507010101_C | Composite of <i>Mytilus edulis</i> |
| MATRIX_CODE | 6181010201 | <i>Homarus americanus</i> |
| MATRIX_CODE | 6181010201_C | Composite of <i>Homarus americanus</i> |
| MATRIX_CODE | 8857041504 | <i>Pseudopleuronectes americanus</i> |
| MATRIX_CODE | 8857041504_C | Composite of <i>Pseudopleuronectes americanus</i> |
| 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 | 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 HOM11 fish and shellfish monitoring. These activities will be overseen by the Normandean Project QA Officer, Mr. Corey Francis.

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. Francis, the Normandean 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 Normandean's "Extraordinary Event Non-Conformity" (EENC) reporting process. The appropriate corrective actions to address any such events will be assessed by Mr. Francis in consultation with Normandean Project Manager, Eric Nestler, and with MWRA. Mr. Francis will generate and/or review all corrective actions required during the project and monitor their effectiveness in meeting project quality objectives. Mr. Nestler will review these issues on a monthly basis, but the QA Director will bring serious issues to Mr. Nestler's attention immediately. Mr. Nestler 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).

Field Corrective Action

Corrective action in the field may be needed when the sample frequency is changed (i.e., more/fewer samples, sample locations other than those specified in this QAPP), or when sampling procedures and/or field analytical procedures require modification due to unexpected conditions. The field team may

identify the need for corrective action. The MWRA 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 | Corey Francis QA Director | Michael Moore Chief Scientist/WHOI | Michael Moore | Corey Francis |
| Lobster Survey | Once, in 2021 | Internal | Normandeau | Corey Francis QA Director | Deborah Rutecki Survey Manager/Normandeau | Deborah Rutecki | Corey Francis |
| Mussel Survey | Once, in 2021 | Internal | Normandeau | Corey Francis QA Director | Erik Fel'Dotto Field Manager/Normandeau | Erik Fel'Dotto | Corey Francis |

| FIELD SAFETY AND EQUIPMENT CHECKLIST | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|--|--|--------------------------|------------|-----------|-------------|--------------------------|--------------------------|--------------|--------------------------|--------------------------|-----------|--------------------------|--------------------------|-------|--------------------------|--------------------------|---------------|--------------------------|--------------------------|--------------------|--------------------------|--------------------------|----------------|--------------------------|--------------------------|-------------|--------------------------|--------------------------|-------------|--------------------------|--------------------------|--------------------|--------------------------|--------------------------|-------------|--------------------------|--------------------------|----------|--------------------------|--------------------------|--------------|--------------------------|--------------------------|-----------|--------------------------|--------------------------|----------|--------------------------|--------------------------|--------------|--------------------------|--------------------------|--------------------|--------------------------|--------------------------|
| <p>FIELD SAFETY CHECKLIST</p> <p>Date of Survey _____</p> <p>Project No. _____</p> <p>Type of work:</p> <ul style="list-style-type: none"> Sample collecting <ul style="list-style-type: none"> Landbased <input type="checkbox"/> Waterbased <input type="checkbox"/> Mooring operations <input type="checkbox"/> Dive operations <input type="checkbox"/> Towed sampling <input type="checkbox"/> Navigation <input type="checkbox"/> Other: _____ <input type="checkbox"/> <p>Type of sample collected:</p> <ul style="list-style-type: none"> Water <input type="checkbox"/> Sediment <input type="checkbox"/> Sludge <input type="checkbox"/> Raw sewerage <input type="checkbox"/> Dredge materials <input type="checkbox"/> Living organisms <input type="checkbox"/> Marine debris <input type="checkbox"/> Electronic data <input type="checkbox"/> Other: _____ <input type="checkbox"/> <p>*Do samples impose a health risk? <input type="checkbox"/> Y <input type="checkbox"/> N</p> <p>If yes, what kind of hazard:</p> <ul style="list-style-type: none"> Chemical <input type="checkbox"/> Y <input type="checkbox"/> N Biological <input type="checkbox"/> Y <input type="checkbox"/> N Radioactive <input type="checkbox"/> Y <input type="checkbox"/> N Other _____ <input type="checkbox"/> Y <input type="checkbox"/> N <p>Specify Hazard: _____</p> <p>* (or fixatives / additives used w/ samples)</p> <p>Is there a spill response plan? <input type="checkbox"/> Y <input type="checkbox"/> N</p> <p>Is one necessary? <input type="checkbox"/> Y <input type="checkbox"/> N</p> <p>Are immunizations necessary? <input type="checkbox"/> Y <input type="checkbox"/> N</p> <p>Will electrical equipment be used by staff? <input type="checkbox"/> Y <input type="checkbox"/> N</p> <p>Will electrical equipment be used on deck? <input type="checkbox"/> Y <input type="checkbox"/> N</p> <p>Will ground fault interrupt (GFI) be used? <input type="checkbox"/> Y <input type="checkbox"/> N</p> <p>Will electrical equipment be checked-out before survey? <input type="checkbox"/> Y <input type="checkbox"/> N</p> <p>List type of sampling equipment to be used: _____</p> <p>Do all members of the survey party have appropriate field experience? <input type="checkbox"/> Y <input type="checkbox"/> N</p> <p>Is training necessary before the survey? <input type="checkbox"/> Y <input type="checkbox"/> N</p> <p>Will there be lifting of heavy objects? <input type="checkbox"/> Y <input type="checkbox"/> N</p> <p>Are all members of survey party familiar with safe lifting practices? <input type="checkbox"/> Y <input type="checkbox"/> N</p> <p>Reviewed and approved _____</p> <p>Task Leader _____ Date _____</p> <p>Chief Scientist _____ Date _____</p> <p>Dept Manager _____ Date _____</p> | <p>FIELD SAFETY EQUIPMENT CHECKLIST</p> <p>Check equipment needed for survey</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 80%;"></th> <th style="width: 10%;">Tech Staff</th> <th style="width: 10%;">Lab Staff</th> </tr> </thead> <tbody> <tr><td>Hard Hats**</td><td><input type="checkbox"/></td><td><input type="checkbox"/></td></tr> <tr><td>Work Vests**</td><td><input type="checkbox"/></td><td><input type="checkbox"/></td></tr> <tr><td>Life Raft</td><td><input type="checkbox"/></td><td><input type="checkbox"/></td></tr> <tr><td>EPIRB</td><td><input type="checkbox"/></td><td><input type="checkbox"/></td></tr> <tr><td>First Aid Kit</td><td><input type="checkbox"/></td><td><input type="checkbox"/></td></tr> <tr><td>Cold Weather Suits</td><td><input type="checkbox"/></td><td><input type="checkbox"/></td></tr> <tr><td>Safety Glasses</td><td><input type="checkbox"/></td><td><input type="checkbox"/></td></tr> <tr><td>Work Gloves</td><td><input type="checkbox"/></td><td><input type="checkbox"/></td></tr> <tr><td>Tyvek Suits</td><td><input type="checkbox"/></td><td><input type="checkbox"/></td></tr> <tr><td>Radiation Detector</td><td><input type="checkbox"/></td><td><input type="checkbox"/></td></tr> <tr><td>Respirators</td><td><input type="checkbox"/></td><td><input type="checkbox"/></td></tr> <tr><td>Air Hood</td><td><input type="checkbox"/></td><td><input type="checkbox"/></td></tr> <tr><td>Face Shields</td><td><input type="checkbox"/></td><td><input type="checkbox"/></td></tr> <tr><td>Lab Coats</td><td><input type="checkbox"/></td><td><input type="checkbox"/></td></tr> <tr><td>Eye Wash</td><td><input type="checkbox"/></td><td><input type="checkbox"/></td></tr> <tr><td>Flash Lights</td><td><input type="checkbox"/></td><td><input type="checkbox"/></td></tr> <tr><td>Spill Response Kit</td><td><input type="checkbox"/></td><td><input type="checkbox"/></td></tr> </tbody> </table> <p>** Required for surveys using vessels</p> <p>Survey Party:</p> <p>_____</p> <p>_____</p> <p>_____</p> <p>_____</p> | | Tech Staff | Lab Staff | Hard Hats** | <input type="checkbox"/> | <input type="checkbox"/> | Work Vests** | <input type="checkbox"/> | <input type="checkbox"/> | Life Raft | <input type="checkbox"/> | <input type="checkbox"/> | EPIRB | <input type="checkbox"/> | <input type="checkbox"/> | First Aid Kit | <input type="checkbox"/> | <input type="checkbox"/> | Cold Weather Suits | <input type="checkbox"/> | <input type="checkbox"/> | Safety Glasses | <input type="checkbox"/> | <input type="checkbox"/> | Work Gloves | <input type="checkbox"/> | <input type="checkbox"/> | Tyvek Suits | <input type="checkbox"/> | <input type="checkbox"/> | Radiation Detector | <input type="checkbox"/> | <input type="checkbox"/> | Respirators | <input type="checkbox"/> | <input type="checkbox"/> | Air Hood | <input type="checkbox"/> | <input type="checkbox"/> | Face Shields | <input type="checkbox"/> | <input type="checkbox"/> | Lab Coats | <input type="checkbox"/> | <input type="checkbox"/> | Eye Wash | <input type="checkbox"/> | <input type="checkbox"/> | Flash Lights | <input type="checkbox"/> | <input type="checkbox"/> | Spill Response Kit | <input type="checkbox"/> | <input type="checkbox"/> |
| | Tech Staff | Lab Staff | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Hard Hats** | <input type="checkbox"/> | <input type="checkbox"/> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Work Vests** | <input type="checkbox"/> | <input type="checkbox"/> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Life Raft | <input type="checkbox"/> | <input type="checkbox"/> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| EPIRB | <input type="checkbox"/> | <input type="checkbox"/> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| First Aid Kit | <input type="checkbox"/> | <input type="checkbox"/> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Cold Weather Suits | <input type="checkbox"/> | <input type="checkbox"/> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Safety Glasses | <input type="checkbox"/> | <input type="checkbox"/> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Work Gloves | <input type="checkbox"/> | <input type="checkbox"/> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Tyvek Suits | <input type="checkbox"/> | <input type="checkbox"/> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Radiation Detector | <input type="checkbox"/> | <input type="checkbox"/> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Respirators | <input type="checkbox"/> | <input type="checkbox"/> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Air Hood | <input type="checkbox"/> | <input type="checkbox"/> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Face Shields | <input type="checkbox"/> | <input type="checkbox"/> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lab Coats | <input type="checkbox"/> | <input type="checkbox"/> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Eye Wash | <input type="checkbox"/> | <input type="checkbox"/> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Flash Lights | <input type="checkbox"/> | <input type="checkbox"/> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Spill Response Kit | <input type="checkbox"/> | <input type="checkbox"/> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Figure 11. Field Safety and Equipment Checklist

Table 15. Example of Field Audit Checklist

| | |
|--|--------------|
| Project: | |
| Site Location: | |
| Auditor: | |
| 1. Was training held for staff not previously trained on project-specific tasks? | |
| 2. Do field personnel have copies of project plan (SAP, QAPP)? | |
| 3. Are samples collected in accordance with the project plan? | |
| 4. Do the numbers and locations of samples conform to the project plan? | |
| 5. Are sample locations in accordance with the project plan? | |
| 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: | |
| Do field personnel have copies of project plan (QAPP)? | |
| 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 three 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 Normandeau, and (3) data collected by Enthalpy. 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 HOM11 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 HOM11 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.

E. REFERENCES

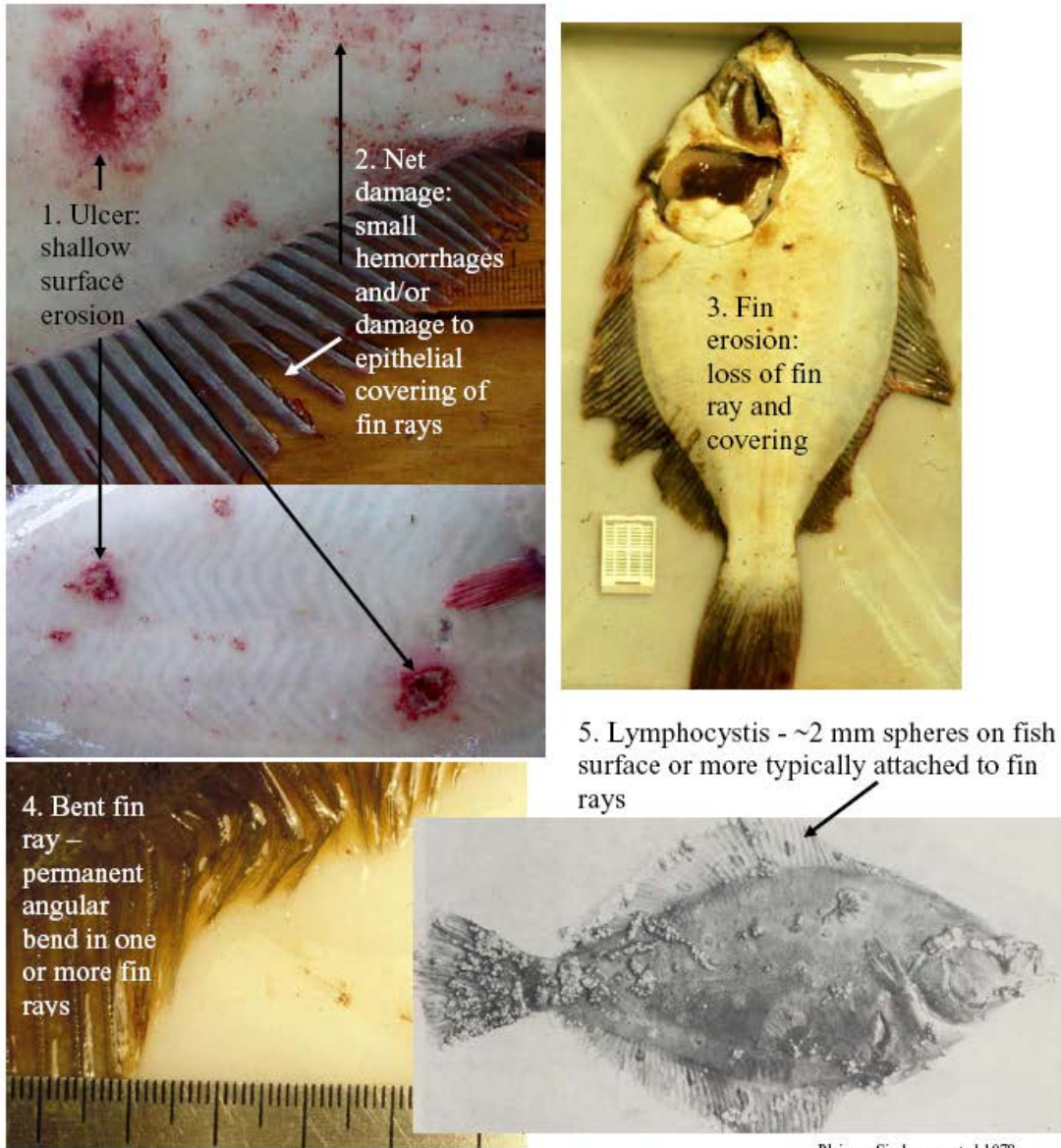
- Fields B. 1988. Chapter 16: Winter flounder *Pseudopleuronectes americanus*. In: Penttila, J and Dery L (eds). Age determination methods for Northwest Atlantic species. NOAA Tech. Rpt. NMFS 72, 135 p.
- Fields B. 1997. Chapter 16: Winter flounder *Pseudopleuronectes americanus*. In: Almeida FP and Sheehan TP (eds). Age determination methods for Northwest Atlantic species. An update of NOAA Tech. Rpt. NMFS 72, 135 p. Accessible at <http://www.nefsc.noaa.gov/fbp/age-man.html>.
- Geoghegan P, Nestler EC, Pembroke AE, Moore MJ. 2016. Quality Assurance Project Plan for Fish and Shellfish Monitoring: 2014-2016, Revision 1. Boston: Massachusetts Water Resources Authority. Report 2016-06. 73 p. plus Appendices A to C.
- Hall M, Lao Y, Moore M, Carroll S. 2010. 2009 fish and shellfish report. Boston: Massachusetts Water Resources Authority. Report 2010-06. 71p.
- Hillman RK, Moore MJ, Peven CS, Lewis DA, Hansen LA, Hunt CD, Stegeman JJ. 1994. 1993 Annual Fish and Shellfish Report. MWRA Environmental Quality Technical Report Series 94-9. Massachusetts Water Resources Authority, Boston MA.
- Lao Y, Constantino J, Leo W, Delaney MF, Epelman P, Rhode S. 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 p.
- Lefkovitz L, Pala SL, Moore MJ. 2004. Combined Work/Quality Assurance Project Plan (CW/QAPP) for Fish and Shellfish Monitoring: 2004-2005. Boston: Massachusetts Water Resources Authority. Report ENQUAD ms-096 Version 1. 34 p.
- Luna L. 1992. Histopathologic methods and color atlas of special stains and tissue artifacts. American Histolabs Gaithersburg, MD.
- Maciolek NJ, Winchell PS, Moore MJ. 2010. Quality Assurance Project Plan (QAPP) for Winter Flounder Monitoring: 2010. Boston: Massachusetts Water Resources Authority. Report 2010-07. 56 pp. plus Appendix A.
- Moore MJ, Stegeman JS. 1993. Liver Pathology of Winter Flounder: Boston Harbor, Massachusetts Bay and Cape Cod Bay - 1992 Report to MWRA Jan 27, 1993.
- Moore MJ, McElroy AE, Geoghegan P, Siskey MR, Pembroke AE. 2016. Flounder Monitoring Report: 2015 Results. Boston: Massachusetts Water Resources Authority. Report 2016-05. 40 p.
- Moore MJ, Nestler EC, Rutecki DA. 2018. Flounder monitoring report: 2018 results. Boston: Massachusetts Water Resources Authority. Report 2018-08. 18 p.
- Moore MJ, Nestler EC, Rutecki DA. 2019. 2019 Flounder monitoring results. Boston: Massachusetts Water Resources Authority. Report 2019-10. 21 p.
- Moore MJ, Pembroke A, Nestler EC, Hall M, Lefkovitz L, Lambert M, Keay K. 2018. Toxics source reduction and sewage upgrades eliminated winter flounder liver neoplasia (1984-2017) from Boston Harbor, MA, USA. *Diseases of Aquatic Organisms* 131:239-243.

- MWRA. 2001. Massachusetts Water Resources Authority Contingency Plan Revision 1. Boston: Massachusetts Water Resources Authority. Report ENQUAD ms-071. 47 p.
- Murchelano RA, Wolke RE. 1985. Epizootic Carcinoma in the Winter Flounder, *Pseudopleuronectes americanus*. Science 3 May 1985:228 (4699): 587–589.
- Nestler EC, Pembroke A, Moore M, Chapnick SD. 2007. 2006 annual fish and shellfish report. Boston: Massachusetts Water Resources Authority. Report ENQUAD 2013-05. 74 p.
- Nestler EC, Pembroke AE, Moore MJ. 2013. Quality Assurance Project Plan for Fish and Shellfish Monitoring: 2011-2013, Revision 1. Boston: Massachusetts Water Resources Authority. Report 2013-05. 74 pp. plus Appendices A to C.
- Normandeau (Normandeau Associates, Inc.). 2018. Mussel bioaccumulation survey report for fish and shellfish monitoring. Task 10, MWRA Harbor and Outfall Monitoring Project. Prepared for Massachusetts Water Resources Authority. October 2018. 6p. + appendix.
- Pembroke A, Nestler E. 2015. Mussel bioaccumulation survey report for fish and shellfish monitoring. Task 10, MWRA Harbor and Outfall Monitoring Project. Prepared for Massachusetts Water Resources Authority. October 2015. 7 p. + appendix.
- Rex AC, Wu D, Coughlin K, Hall M, Key KE, Taylor DI. 2002. The State of Boston Harbor: Mapping the Harbor's Recovery. MWRA Environmental Quality Technical Report Series 2002-09. Massachusetts Water Resources Authority, Boston MA. 44 p.
- Rutecki D, Nestler EC, Moore MJ. 2017. Quality Assurance Project Plan for Fish and Shellfish Monitoring: 2017-2019. Boston: Massachusetts Water Resources Authority. Report 2017-03. 73 p. plus Appendices A to C.
- EPA. (U.S. Environmental Protection Agency). 2001 (reissued 2006). EPA Requirements for Quality Assurance Project Plans, March 2001, (EPA QA/R-5), EPA/240/B-01/003, United States Environmental Protection Agency, Washington, D.C. <http://www.epa.gov/quality/qs-docs/r5-final.pdf>.
- EPA. (U.S. Environmental Protection Agency). 2002. EPA Guidance for Quality Assurance Project Plans, December 2002, (EPA QA/G-5), EPA/240/R-02/009, United States Environmental Protection Agency, Washington, D.C.
- Werme C, Hunt CD. 2008. Outfall monitoring overview BACKGROUND. Boston: Massachusetts Water Resources Authority. Report 2008-18. 54p.

Appendix A

Guidance for Recording External Lesions in Flounder

SCORING EXTERNAL LESIONS ON WINTER FLOUNDER



Plaice - Sinderman et al 1978

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

Appendix B

Flounder Tissue Resection SOP

Standard Operating Protocol for Dissection of Winter Flounder following FF201 Flounder Survey for Normandeau/ Enthalpy

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:

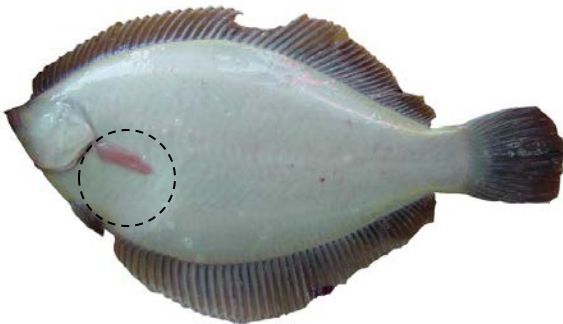
Cooler with ice and individually bagged and tagged flounder
Histology cassettes
Cassette marking pencil
4 screw top jars of buffered formalin (one per station)
Data sheet with field data/ Chain of custody form
Titanium forceps and blade to dissect out liver.
Rubber bands to ensure cassettes do not open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

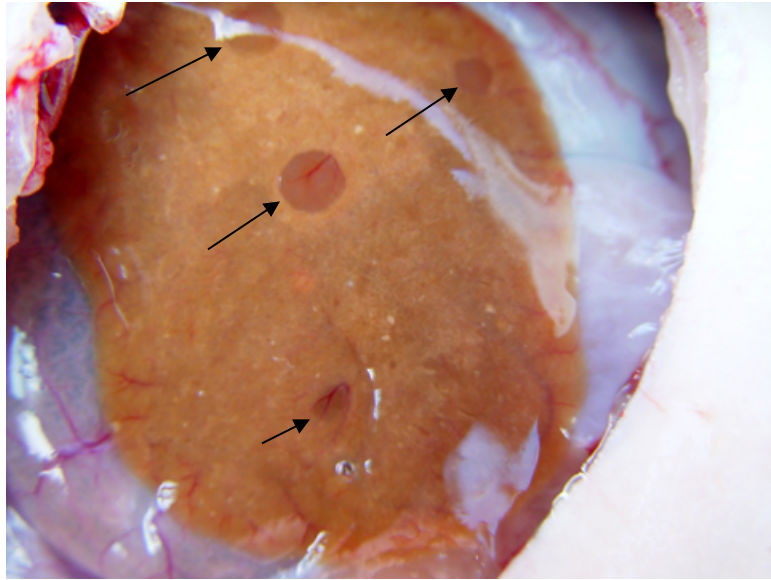
Clean all instruments
Open blind side of visceral cavity – see dotted line below



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, Y-YB: Yellow-Y 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

Safety Data Sheet

Formaldehyde 37% M 11%

Section 1 - Product and Company Identification

GHS product identifier: Formaldehyde 37% M 11 %

MSDS Number: 000000100073

Product type: Formaldehyde Solution

Material uses: Industrial use.

Manufacturer/Supplier/Importer: Hexion Canada, Inc.
180 East Broad Street
Columbus, Ohio
43215 USA

Contact person: 4information@hexion.com

Telephone: For additional health and safety or regulatory information, call 1 888 443 9466.

Emergency telephone number: For Emergency Medical Assistance
Call Health & Safety Information Services
1-866-303-6949

For Emergency Transportation Information
CHEMTREC US Domestic (800) 424-9300
CHEMTREC International (703) 527-3887
CANUTEC CA Domestic (613) 996-6666

Section 2 – Hazards Identification

Classification of the substance or mixture:

FLAMMABLE LIQUIDS - Category 4
ACUTE TOXICITY: oral - Category 4
ACUTE TOXICITY: dermal - Category 3
ACUTE TOXICITY: inhalation - Category 3
SKIN CORROSION/IRRITATION - Category 2
SERIOUS EYE DAMAGE/EYE IRRITATION - Category I
RESPIRATORY SENSITIZATION - Category I
SKIN SENSITIZATION - Category I
CARCINOGENICITY - Category I B
TOXIC TO REPRODUCTION - Category 1 B
TOXIC TO REPRODUCTION - Category I B
SPECIFIC TARGET ORGAN TOXICITY (SINGLE EXPOSURE) [central nervous system (CNS)]- Category I
SPECIFIC TARGET ORGAN TOXICITY (REPEATED EXPOSURE) [respiratory tract, gastrointestinal tract, skin, kidneys] -Category 2

GHS label elements**Hazard pictograms:****Signal word/Hazard statements:**

Danger
H227 Combustible liquid.
H302 Harmful if swallowed.
H311 Toxic in contact with skin.
H331 Toxic if inhaled.
H315 Causes skin irritation.
H318 Causes serious eye damage.
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H317 May cause an allergic skin reaction.
H350 May cause cancer.
H360F May damage fertility.
H360 May damage the unborn child.
H370 Causes damage to organs: (central nervous system (CNS))
H373 May cause damage to organs through prolonged or repeated exposure (respiratory tract, gastrointestinal tract, skin, kidneys)

Precautionary statements

General: Not applicable.

Prevention: Obtain special instructions before use. Do not handle until all safety precautions have been read and understood. Use personal protective equipment as required. Wear protective gloves. Wear eye or face protection. Wear protective clothing. In case of inadequate ventilation wear respiratory protection. Keep away from flames and hot surfaces. No smoking. Use only outdoors or in a well-ventilated area. Do not breathe vapor. Do not eat, drink or smoke when using this product. Wash hands thoroughly after handling. Contaminated work clothing should not be allowed out of the workplace.

Response: Get medical attention if you feel unwell.

IF exposed: Call a POISON CENTER or physician.

IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. Call a POISON CENTER or physician. If experiencing respiratory symptoms: Call a POISON CENTER or physician.

IF SWALLOWED: Call a POISON CENTER or physician if you feel unwell. Rinse mouth.

IF ON SKIN: Take off immediately all contaminated clothing. Wash with plenty of soap and water. Call a POISON CENTER or physician if you feel unwell. Take off contaminated clothing. Wash contaminated clothing before reuse. If skin irritation or rash occurs: Get medical attention.

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or physician.

Storage: Store locked up. Store in a well-ventilated place. Keep cool.

Disposal: Dispose of contents and container in accordance with all local, regional, national and international regulations.

Other hazards which do not result in classification: None known.

Section 3 – Composition/Information on Ingredients

Substance/mixture: Mixture

| Ingredient name | % by weight | CAS number |
|-----------------|-------------|------------|
| Formaldehyde | 35 - 50 | 50-00-0 |
| Methanol | 10 - 12.5 | 67-56-1 |

There are no additional ingredients present which, within the current knowledge of the supplier and in the concentrations applicable, are classified as hazardous to health or the environment and hence require reporting in this section.

Occupational exposure limits, if available, are listed in Section 8.

Section 4 - First Aid Measures

Description of necessary first aid measures

Eye contact: Get medical attention immediately. Call a poison center or physician. Immediately flush eyes with plenty of water, occasionally lifting the upper and lower eyelids. Check for and remove any contact lenses. Continue to rinse for at least 10 minutes. Chemical burns must be treated promptly by a physician.

Inhalation: Get medical attention immediately. Call a poison center or physician. Remove victim to fresh air and keep at rest in a position comfortable for breathing. If it is suspected that fumes are still present, the rescuer should wear an appropriate mask or self-contained breathing apparatus. If not breathing, if breathing is irregular or if respiratory arrest occurs, provide artificial respiration or oxygen by trained personnel. It may be dangerous to the person providing aid to give mouth-to-mouth resuscitation. If unconscious, place in recovery position and get medical attention immediately. Maintain an open airway. Loosen tight clothing such as a collar, tie, belt or waistband. In the event of any complaints or symptoms, avoid further exposure.

Skin contact: Get medical attention immediately. Call a poison center or physician. Wash with plenty of soap and water. Remove contaminated clothing and shoes. Wash contaminated clothing thoroughly with water before removing it, or wear gloves. Continue to rinse for at least 10 minutes. Chemical burns must be treated promptly by a physician. In the event of any complaints or symptoms, avoid further exposure. Wash clothing before reuse. Clean shoes thoroughly before reuse.

Ingestion: Get medical attention immediately. Call a poison center or physician. Wash out mouth with water. Remove dentures if any. Remove victim to fresh air and keep at rest in a position comfortable for breathing. If material has been swallowed and the exposed person is conscious, give small quantities of water to drink. Stop if the exposed person feels sick as vomiting may be dangerous. Do not induce vomiting unless directed to do so by medical personnel. If vomiting occurs, the head should be kept low so that vomit does not enter the lungs. Chemical burns must be treated promptly by a physician. Never give anything by mouth to an unconscious person. If unconscious, place in recovery position and get medical attention immediately. Maintain an open airway. Loosen tight clothing such as a collar, tie, belt or waistband.

Indication of immediate medical attention and special treatment needed, if necessary

Notes to physician: Treat symptomatically. Contact poison treatment specialist immediately if large quantities have been ingested or inhaled.

Specific treatments: No specific treatment.

Protection of first aid personnel: No action shall be taken involving any personal risk or without suitable training. If it is suspected that fumes are still present, the rescuer should wear an appropriate mask or self-contained breathing apparatus. It may be dangerous to the person providing aid to give mouth-to-mouth resuscitation. Wash contaminated clothing thoroughly with water before removing it, or wear gloves.

See toxicological information (Section 11)

Section 5 - Fire Fighting Measures

Extinguishing media

Suitable extinguishing media: Use dry chemical, CO₂, water spray (fog) or foam.

Unsuitable extinguishing media: Do not use water jet.

Specific hazards arising from the chemical: Combustible liquid. In a fire or if heated, a pressure increase will occur and the container may burst, with the risk of a subsequent explosion. Runoff to sewer may create fire or explosion hazard.

Hazardous thermal decomposition products: Decomposition products may include the following materials: carbon dioxide, carbon monoxide, and metal oxide/oxides.

Special protective actions for firefighters: Promptly isolate the scene by removing all persons from the vicinity of the incident if there is a fire. No action shall be taken involving any personal risk or without suitable training. Move containers from fire area if this can be done without risk. Use water spray to keep fire exposed containers cool.

Special protective equipment for fire-fighters: Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face -piece operated in positive pressure mode.

Section 6 - Accidental Release Measures

Personal precautions, protective equipment and emergency procedures

For non-emergency personnel: No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Shut off all ignition sources. No flares, smoking or flames in hazard area. Do not breathe vapor or mist. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment.

For emergency responders: If specialized clothing is required to deal with the spillage, take note of any information in Section 8 on suitable and unsuitable materials. See also the information in "For non-emergency personnel".

Environmental precautions: Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).

Methods and material for containment and cleaning up

Small spill: Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble. Alternatively, or if water-insoluble, absorb with an inert dry material and place in an appropriate waste disposal container. Use spark-proof tools and explosion-proof equipment. Dispose of via a licensed waste disposal contractor.

Large spill: Stop leak if without risk. Move containers from spill area. Approach release from upwind. Prevent entry into sewers, water courses, basements or confined areas. Wash spillages into an effluent treatment plant or proceed as follows. Contain and collect spillage with noncombustible, absorbent material e.g. sand, earth, vermiculite or diatomaceous earth and place in container for disposal according to local regulations (see section 13 of SDS). Use spark-proof tools and explosion-proof equipment. Dispose of via a licensed waste disposal contractor. Contaminated absorbent material may pose the same hazard as the spilled product. Note: see section 1 of SDS for emergency contact information and section 13 of SDS for waste disposal.

Section 7 - Handling and Storage

Precautions for safe handling

Protective measures: Put on appropriate personal protective equipment (see section 8 of SDS). Persons with a history of skin sensitization problems or asthma, allergies or chronic or recurrent respiratory disease should not be employed in any process in which this product is used. Avoid exposure - obtain special instructions before use. Avoid exposure during pregnancy. Do not handle until all safety precautions have been read and understood. Do not get in eyes or on skin or clothing. Do not breathe vapor or mist. Do not ingest. Use only with adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Do not enter storage areas and confined spaces unless adequately ventilated. Keep in the original container or an approved alternative made from a compatible material, kept tightly closed when not in use. Store and use away from heat, sparks, open flame or any other ignition source. Use explosion-proof electrical (ventilating, lighting and material handling) equipment. Use only non-sparking tools. Empty containers retain product residue and can be hazardous. Do not reuse container. Take precautionary measures against electrostatic discharges. To avoid fire or explosion, dissipate static electricity during transfer by grounding and bonding containers and equipment before transferring material. Follow US NFPA 30, "Flammable & Combustible Liquids Code," or other national, state and local codes on safe handling of flammable liquids. Train workers in the recognition and prevention of hazards associated with the storage, handling and transfer of flammable liquids in the plant. Store in an area designated for storage of flammable liquids (See NPFA 30, and OSHA 29 CFR 1910.106)

Advice on general occupational hygiene: Eating, drinking and smoking should be prohibited in areas where this material is handled, stored and processed. Workers should wash hands and face before eating, drinking and smoking. Remove contaminated clothing and protective equipment before entering eating areas. See also Section 8 for additional information on hygiene measures.

Conditions for safe storage, including any incompatibilities: Store in original container protected from direct sunlight in a dry, cool and well-ventilated area, away from incompatible materials (see section 10 of SDS) and food and drink. Store locked up. Eliminate all ignition sources. Separate from oxidizing materials. Keep container tightly closed and sealed until ready for use. Containers that have been opened must be carefully resealed and kept upright to prevent leakage. Do not store in unlabeled containers. Use appropriate containment to avoid environmental contamination.

Section 8 - Exposure Controls, Personal Protection

Control parameters

Occupational exposure limits

| Ingredient name | Exposure limits |
|-----------------|---|
| Formaldehyde | <p>ACGIH TLV (2000-03-01) Ceiling 0.37 mg/m³ 0.3 ppm</p> <p>OSHA PEL (1993-06-30) Time Weighted Average (TWA) 0.75 ppm</p> <p>Pollutant concentration that should not be exceeded during working hours and which workers are believed to be exposed during a period of 15 minutes maximum, without experiencing: a) irritation. b) chronic or irreversible tissue damage. c) dependent toxic effects of exposure rate. d) Narcosis of sufficient magnitude to increase susceptibility to accidents. e) The reduction of ability to get to safety by their own means.</p> <p>2 ppm</p> <p>NIOSH REL (1994-06-01) Time Weighted Average (TWA) 0.016 ppm Ceiling 0.1 ppm</p> |
| Methanol | <p>ACGIH TLV (1994-09-01) Time Weighted Average (TWA) 262 mg/m³ 200 ppm</p> <p>Short Term Exposure Limit (STEL) 328 mg/m³ 250 ppm</p> <p>OSHA PEL (1993-06-30) Time Weighted Average (TWA) 260 mg/m³ 200 ppm</p> <p>NIOSH REL (1994-06-01) Time Weighted Average (TWA) 260 mg/m³ 200 ppm</p> <p>Pollutant concentration that should not be exceeded during working hours and which workers are believed to be exposed during a period of 15 minutes maximum, without experiencing: a) irritation. b) chronic or irreversible tissue damage. c) dependent toxic effects of exposure rate. d) Narcosis of sufficient magnitude to increase susceptibility to accidents. e) The reduction of ability to get to safety by their own means.</p> <p>325 mg/m³ 250 ppm</p> |

Recommended monitoring procedures: If this product contains ingredients with exposure limits, personal, workplace atmosphere or biological monitoring may be required to determine the effectiveness of the ventilation or other control measures and/or the necessity to use respiratory protective equipment. Reference should be made to appropriate monitoring standards. Reference to national guidance documents for methods for the determination of hazardous substances will also be required.

Appropriate engineering controls: Use only with adequate ventilation. Use process enclosures, local exhaust ventilation or other engineering controls to keep worker exposure to airborne contaminants below any recommended or statutory limits. The engineering controls also need to keep gas, vapor or dust concentrations below any lower explosive limits. Use explosion-proof ventilation equipment.

Environmental exposure controls: Emissions from ventilation or work process equipment should be checked to ensure they comply with the requirements of environmental protection legislation. In some cases, fume scrubbers, filters or engineering modifications to the process equipment will be necessary to reduce emissions to acceptable levels.

Individual protection measures

Hygiene measures: Wash hands, forearms and face thoroughly after handling chemical products, before eating, smoking and using the lavatory and at the end of the working period. Appropriate techniques should be used to remove potentially contaminated clothing. Contaminated work clothing should not be allowed out of the workplace. Wash contaminated clothing before reusing. Ensure that eyewash stations and safety showers are close to the workstation location.

Eye/face protection: Safety eyewear complying with an approved standard should be used when a risk assessment indicates this is necessary to avoid exposure to liquid splashes, mists, gases or dusts. If contact is possible, the following protection should be worn, unless the assessment indicates a higher degree of protection: chemical splash goggles and/or face shield. If inhalation hazards exist, a full-face respirator may be required instead.

Skin protection

Hand protection: Chemical-resistant, impervious gloves complying with an approved standard should be worn at all times when handling chemical products if a risk assessment indicates this is necessary. Considering the parameters specified by the glove manufacturer, check during use that the gloves are still retaining their protective properties. It should be noted that the time to breakthrough for any glove material may be different for different glove manufacturers. In the case of mixtures, consisting of several substances, the protection time of the gloves cannot be accurately estimated.

Body protection: Personal protective equipment for the body should be selected based on the task being performed and the risks involved and should be approved by a specialist before handling this product.

Other skin protection: Appropriate footwear and any additional skin protection measures should be selected based on the task being performed and the risks involved and should be approved by a specialist before handling this product.

Respiratory protection: Use a properly fitted, air-purifying or air-fed respirator complying with an approved standard if a risk assessment indicates this is necessary. Respirator selection must be based on known or anticipated exposure levels, the hazards of the product and the safe working limits of the selected respirator.

Section 9 - Physical and Chemical Properties

Appearance

Physical State: Liquid
Color: Clear, colorless/colourless
Odor: none reported
Odor threshold: Not available
pH: 3.0 - 4.5.
Melting point/Freezing point: See storage section
Boiling Point: 100 °C (212.00 °F)
Flash Point: Tag Closed Cup: 62 °C (143.60 °F) (ASTM D 56)
Burning time: Not available
Burning rate: Not available
Evaporation rate: 1 ((n-Butyl acetate= 1))
Flammability (solid, gas): Not available
Lower and upper explosive (flammable) limits: Lower: 7 %(V); Upper: 70 %(V)
Vapor Pressure: 40 mm Hg @ 39 °C (102.20 OF).
Vapor Density: 1 [Air = 1).
Relative density: 1.0820 - 1.0880
Solubility: Not available
Solubility in water: Infinite
Partition coefficient: n-octano/water: Not available
Auto-ignition temperature: 420 °C (788.00 OF)
Decomposition temperature: Not available
SADT: Not available
Viscosity: Dynamic: Not available, **Kinematic:** Not available
Other information: No additional information

Section 10 - Stability and Reactivity

Reactivity: Stable under normal conditions.

Chemical stability: The product is stable.

Possibility of hazardous reactions: Under normal conditions of storage and use, hazardous reactions will not occur.

Conditions to avoid: Avoid all possible sources of ignition (spark or flame). Do not pressurize, cut, weld, braze, solder, drill, grind or expose containers to heat or sources of ignition.

Incompatible materials: Reactive or incompatible with the following materials: oxidizing materials, strong acids, strong alkalis, phenol, HYDROCHLORIC ACID.

Hazardous decomposition products: Decomposition products may include the following materials: carbon dioxide, carbon monoxide. Under normal conditions of storage and use, hazardous decomposition products should not be produced.

Other hazards: May further react at high temperatures to form methanol, formic acid or methylals. At low temperatures will self-polymerize to form paraformaldehyde.

Section 11 - Toxicological Information

Information on toxicological effects

Acute toxicity

| Product/ingredient name | Result | Species | Dose | Exposure |
|-------------------------|-----------------|---------|-------------|----------|
| Formaldehyde | | | | |
| | LD50 Oral | Rat | 800 mg/kg | - |
| | LC50 Inhalation | Rat | 0.578 mg/l | 2 h |
| | LD50 Dermal | Rabbit | 270 mg/kg | - |
| Methanol | | | | |
| | LD50 Oral | Rat | 5,628 mg/kg | - |

Conclusion/Summary: Not available

Irritation/Corrosion

| Product/ingredient name | Result | Species | Score | Exposure | Observation |
|-------------------------|-------------------------|---------|-------|----------|-------------|
| Formaldehyde | Skin - Erythema/E schar | Rabbit | 2.5 | 20 hrs | - |
| | Skin - Edema | Rabbit | 3 | 20 hrs | - |
| | eyes - Cornea opacity | Mouse | > 3 | | - |

Conclusion/Summary:

Skin: Not available

Eyes: Not available

Respiratory: Not available

Sensitization

Skin: Not available

Respiratory: Not available

Mutagenicity:

Conclusion/Summary: Not available

Carcinogenicity

| Product/ingredient name | Result | Species | Dose | Exposure |
|-------------------------|--|---------|------|----------|
| Formaldehyde | ----- | - | | |
| Remarks: | The National Toxicology Program (NTP) classifies formaldehyde as “known to be a human carcinogen” with respect to nasopharyngeal cancer, sinonasal cancer and myeloid leukemia. The International Agency for Research on Cancer (IARC) classifies formaldehyde as “carcinogenic to humans”. U.S. OSHA regulates formaldehyde as a potential human carcinogen. See the OSHA Formaldehyde Workplace Standard at 29 CFR 1920.1048 (the “OSHA Standard”). Safe handling and use instructions are provided in this SDS and in the OSHA Standard. OSHA has identified 0.5 ppm, calculated as an eight-hour time-weighted average (“TWA”) concentration, as the “Action Level”. Please review and understand the guidance contained in this MSDS, and refer to the OSHA Standard for regulatory requirements that might be applicable to your operation and use. Many studies and other evaluations have been performed concerning formaldehyde’s potential to cause cancer. To review some of these studies and for further information go to www.osha.gov/SLTC/formaldehyde ; http://monographs.iarc.fr ; http://ntp-server.niehs.nih.gov ; http://epa.gov/iris/subst/0419.htm ; http://www.nap.edu/catalog.php?record_id=13142 and other authoritative websites. | | | |

Conclusion/Summary: Not available

Reproductive toxicity

Conclusion/Summary: Not available

Teratogenicity

Conclusion/Summary: Not available

Specific target organ toxicity (single exposure)

| Product/ingredient name | Category | Route of exposure | Target organs |
|-------------------------|--|-------------------|---|
| Formaldehyde | Category 3 | | Respiratory tract irritation |
| Methanol | Category 3 Category 1 Category 2 | | Respiratory tract irritation central nervous system (CNS) optic nerve |

Specific target organ toxicity (repeat exposure)

| Product/ingredient name | Category | Route of exposure | Target organs |
|-------------------------|------------|-------------------|---|
| Formaldehyde | Category 2 | | respiratory tract skin |
| Methanol | Category 2 | | kidneys liver gastrointestinal tract skin respiratory tract |

Aspiration hazard: Not available

Information on the likely routes of exposure: Not available

Potential acute health effects

Eye contact: Causes serious eye damage.

Inhalation: Toxic if inhaled. May give off gas, vapor or dust that is very irritating or corrosive to the respiratory system. May cause allergy or asthma symptoms or breathing difficulties if inhaled.

Skin contact: Toxic in contact with skin. Causes skin irritation. May cause an allergic skin reaction.

Ingestion: Harmful if swallowed. May cause burns to mouth, throat and stomach.

Symptoms related to the physical, chemical and toxicological characteristics

Eye contact: Adverse symptoms may include the following: pain, watering, and redness.

Inhalation: Adverse symptoms may include the following: wheezing and breathing difficulties, asthma, reduced fetal weight, increase in fetal deaths, and skeletal malformations.

Skin contact: Adverse symptoms may include the following: pain or irritation, redness, blistering, reduced fetal weight, increase in fetal deaths, and skeletal malformations.

Ingestion: Adverse symptoms may include the following: stomach pains, reduced fetal weight, increase in fetal deaths, and skeletal malformations.

Delayed and immediate effects and also chronic effects from short and long term exposure

Short term exposure

Potential immediate effects: Not available

Potential delayed effects: Not available

Long term exposure

Potential immediate effects: Not available

Potential delayed effects: Not available

Potential chronic health effects

Conclusion/Summary: Not available

General: May cause damage to organs through prolonged or repeated exposure. Once sensitized, a severe allergic reaction may occur when subsequently exposed to very low levels.

Carcinogenicity: May cause cancer. Risk of cancer depends on duration and level of exposure.

Mutagenicity: No known significant effects or critical hazards.

Teratogenicity: May damage the unborn child.

Developmental effects: No known significant effects or critical hazards.

Fertility effects: May damage fertility.

Numerical measures of toxicity

Acute toxicity estimates

| | |
|---------------------|------------------|
| Route | ATE value |
| Oral | 618.8 mg/kg |
| Route | ATE value |
| Dermal | 566.7 mg/kg |
| Route | ATE value |
| Inhalation (vapors) | 8.044 mg/l |

Section 12 - Ecological Information

Toxicity

| Product/ingredient name | Result | Species | Exposure |
|-------------------------|------------------------------------|---------------------------------------|----------|
| formaldehyde | | | |
| | Acute LC50 6.7 mg/l - | Fish - Striped bass | 96 h |
| | Acute LC50 6.9 mg/l - | Fish - Zebra danio | 6 d |
| | Acute NOEC > 47.9 mg/l - | Fish - Medaka, high-eyes | 28 d |
| | Acute EC50 5.8 mg/l Fresh water | Aquatic invertebrates. Water flea | 2 d |
| | Acute EC50 4.9 mg/l Fresh water | Aquatic plants - Algae | 72 h |
| | Acute EC50 4.3 mg/l Fresh water | Aquatic plants - Algae | 48 h |
| | Acute EC50 19 mg/l - | Micro-organism - Soil organisms | 3 h |
| methanol | | | |
| | Acute EC50 13,000 mg/l Fresh water | Fish - Rainbow trout, donaldson trout | 4 d |

Conclusion/Summary: Not available

Persistence/degradability

Conclusion/Summary: Not available

Bioaccumulative potential

| Product/ingredient name | LogPow | BCF | Potential |
|-------------------------|--------|-----|-----------|
| Formaldehyde | 0.35 | < 1 | low |
| Methanol | -0.77 | - | low |

Mobility in soil

Soil/water partition coefficient (KOC): Not available

Other adverse effects: No known significant effects or critical hazards.

Section 13 - Disposal Considerations

Disposal methods: The generation of waste should be avoided or minimized wherever possible. Disposal of this product, solutions and any by-products should at all times comply with the requirements of environmental protection and waste disposal legislation and any regional local authority requirements. Dispose of surplus and non-recyclable products via a licensed waste disposal contractor. Waste should not be disposed of untreated to the sewer unless fully compliant with the requirements of all authorities with jurisdiction. Waste packaging should be recycled. Incineration or landfill should only be considered when recycling is not feasible. This material and its container must be disposed of in a safe way. Care should be taken when handling emptied containers that have not been cleaned or rinsed out. Empty containers or liners may retain some product residues. Vapor from product residues may create a highly flammable or explosive atmosphere inside the container. Do not cut, weld or grind used containers unless they have been cleaned thoroughly internally. Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers.

Section 14 - Transport Information

The data provided in this section is for information only and may not be specific to your package size or mode of transport. You will need to apply the appropriate regulations to properly classify your shipment for transportation.

International transport regulations

| Regulatory information | UN/NA number | Proper shipping name | Classes/*PG | Reportable Quantity (RQ) |
|------------------------|--------------|-----------------------|-------------|--------------------------|
| CFR | 2209 | FORMALDEHYDE SOLUTION | Class 8 III | Formaldehyde, Methanol |
| TDG | 2209 | FORMALDEHYDE SOLUTION | Class 8 III | |
| IMO/IMDG | 2209 | FORMALDEHYDE SOLUTION | Class 8 III | |
| IATA (Cargo) | 2209 | FORMALDEHYDE SOLUTION | Class 8 III | |

*PG : Packing group

Special precautions for user: Transport within user's premises: always transport in closed containers that are upright and secure. Ensure that persons transporting the product know what to do in the event of an accident or spillage.

Section 15 - Regulatory Information

United States

US FEDERAL

United States - TSCA 12(b) - Chemical export notification: None required.

United States - TSCA 5(a)2 - Final significant new use rules: Not listed

United States - TSCA 5(a)2 - Proposed significant new use rules: Not listed

SARA 313

| | | Product name | CAS number |
|--|---|--------------|------------|
| Form R - Reporting requirements | : | Formaldehyde | 50-00-0 |
| | : | Methanol | 67-56-1 |
| Supplier notification | : | Formaldehyde | 50-00-0 |
| | : | Methanol | 67-56-1 |

SARA 313 notifications must not be detached from the MSDS and any copying and redistribution of the MSDS shall include copying and redistribution of the notice attached to copies of the MSDS subsequently redistributed.

California Prop 65

The following statement is made in order to comply with the California Safe Drinking Water Act: WARNING: This product contains a chemical known to the State of California to cause cancer and birth defects or other reproductive harm.

United States inventory (TSCA 8b): All components are listed or exempted.

Canada

WHMIS (Canada): Class B-3: Combustible liquid with a flash point between 37.8°C (100°F) and 93.3°C (200°F).

Class D-1A: Material causing immediate and serious toxic effects (Very toxic).

Class D-2A: Material causing other toxic effects (Very toxic).

Class D-28: Material causing other toxic effects (Toxic).

Class E: Corrosive material

Canadian lists:

Canadian NPRI: The following components are listed: Formaldehyde Methanol

CEPA Toxic substances: The following components are listed: Formaldehyde

International regulations

International lists: Philippines inventory (PICCS): All components are listed or exempted.

New Zealand Inventory (NZIoC): Not determined.

Korea inventory: All components are listed or exempted.

Japan inventory: All components are listed or exempted.

China inventory (IECSC): All components are listed or exempted.

Australia inventory (AICS): All components are listed or exempted.

Canada inventory: All components are listed or exempted.

United States inventory (TSCA 8b): All components are listed or exempted.

New Zealand Inventory (NZIoC): All components are listed or exempted.

Taiwan inventory (CSNN): All components are listed or exempted.

Section 16 - Additional Information

Hazardous Material Information System III (U.S.A.) :

| | | |
|------------------|---|---|
| Health | * | 3 |
| Flammability | | 2 |
| Physical hazards | | 0 |
| | | |

Caution: HMIS® ratings are based on a 0-4 rating scale, with 0 representing minimal hazards or risks, and 4 representing significant hazards or risks. Although HMIS® ratings are not required on MSDSs under 29 CFR 1910.1200, the preparer may choose to provide them. HMIS® ratings are to be used with a fully implemented HMIS® program. HMIS® is a registered mark of the National Paint & Coatings Association (NPCA). HMIS® materials may be purchased exclusively from J. J. Keller (800) 327-6868. The customer is responsible for determining the PPE code for this material.

Full text of abbreviated H statements: Not applicable.

Date of issue/Date of revision: 05/26/2015

Prepared by: Product Safety Stewardship

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 |
| Qualifier for WEIGHT | WEIGHT_QUAL | | alphanumeric, maximum 4 characters |

| Description | Field | Required Field | Data type & format |
|--|-----------------|----------------|--------------------------------------|
| 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 |

| Description | Field | Required Field | Data type & format |
|--|--------------------------|----------------|--------------------------------------|
| Identifier for sub-sample bottle (generally corresponds to number on label). | BOTTLE_ID | Y | alphanumeric, maximum 15 characters |
| Apoptotic 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 morphology 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) | 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 FF21110C1, FF21110C2, and FF21110C3.

| EVENT_ID | SAMPLE_ID | SAMPLEC_ID |
|----------|-----------|------------|
| FF211 | FF2111001 | FF21110C1 |
| FF211 | FF2111002 | FF21110C1 |
| FF211 | FF2111003 | FF21110C1 |
| FF211 | FF2111004 | FF21110C1 |
| FF211 | FF2111005 | FF21110C1 |
| FF211 | FF2111006 | FF21110C2 |
| FF211 | FF2111007 | FF21110C2 |
| FF211 | FF2111008 | FF21110C2 |
| FF211 | FF2111009 | FF21110C2 |
| FF211 | FF2111010 | FF21110C2 |
| FF211 | FF2111011 | FF21110C3 |
| FF211 | FF2111012 | FF21110C3 |
| FF211 | FF2111013 | FF21110C3 |
| FF211 | FF2111014 | FF21110C3 |
| FF211 | FF2111015 | FF21110C3 |

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 |



Massachusetts Water Resources Authority
100 First Avenue • Boston, MA 02129
www.mwra.com
617-242-6000