Technical Reports

Massachusetts Division of Marine Fisheries
Technical Report TR-73

Quality Assurance Program Plan (QAPP) for Water Quality Measurements during Diadromous Fish Monitoring

Version 2.0

B. C. Chase, J. J. Sheppard, B. I. Gahagan, and S. M. Turner

Massachusetts Division of Marine Fisheries
Department of Fish and Game
Executive Office of Energy and Environmental Affairs
Commonwealth of Massachusetts

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Quality Assurance Program Plan (QAPP) for Water Quality Measurements during Diadromous Fish Monitoring

Version 2.0

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June 2020

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Kathleen Theoharides, Secretary
Department of Fish and Game
Ronald S. Amidon, Commissioner
Massachusetts Division of Marine Fisheries
Daniel J. McKiernan, Director

Title: Quality Assurance Program Plan for Water Quality Measurements conducted for Diadromous Fish Monitoring (Version 2.0)

Date: March 2, 2020

Program Participant: Massachusetts Division of Marine Fisheries

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Abstract

Diadromous fish migrate between freshwater and marine habitats to complete essential life history stages. In New England, this group of fish is represented by anadromous species such as river herring that mature in the ocean and undergo spring spawning runs to freshwater habitat, and by a single catadromous fish, the American eel, that spawn at sea and the young migrate to freshwater habitat to reside until maturation. The origin of diadromy is not certain, but likely evolved as a selective advantage in the form of higher survival over a life history that did not switch habitats. Most anadromous fish in New England have a demersal or semi-demersal egg that incubates in contact with the substrata where spawning occurred. The success of this reproductive strategy is dependent on elevated spring flows and suitable water and habitat quality. Diadromous fish populations on the Atlantic coast of North America have declined in recent decades. The causes for the declines are under investigation; with watershed alterations, harvest mortality, and passage impediments known to be negative influences in most regions. It is reasonable to expect that reductions in water and habitat quality at spawning and nursery habitats could reduce the adaptive advantage of these migrations and chronically impact population recruitment.

Interest in the status of diadromous fish has fortunately increased recently among constituents and government agencies. The majority of restoration efforts have focused on migratory impediments with less attention on water and habitat quality. The Massachusetts Division of Marine Fisheries (DMF) developed a quality assurance program plan (QAPP) for water quality measurements during diadromous fish monitoring to provide standardized sampling protocols in order to improve the traceability and reliability of water quality data. Standard Operating Procedures (SOP) are provided for routine measurements and multi-project applications for water temperature and chemistry loggers and for river herring and rainbow smelt habitat assessments. The habitat assessment data will also provide guidance for diadromous fish restoration efforts. The QAPP was designed to coordinate sampling efforts to produce data that is acceptable and contributes to Waterbody Assessments (Clean Water Act , Section 305 (b)) and aquatic resource protection and restoration efforts under CWA Section 303 (d) conducted by the Massachusetts Department of Environmental Protection (MassDEP).

Outside of minor updates and improvements, the primary changes made in this QAPP version (2.0) from the first version published in 2010 are the addition of a new SOP on fish kill investigations, protocols for sampling with the YSI 556 water chemistry instrument in SOP 2.0, and refinements to SOP 4.0 on river herring spawning and nursery habitat assessment.

Table of Contents

Standard Operating Procedures

INTRODUCTION

This quality assurance program plan (QAPP) addresses water quality measurements and analysis for monitoring projects conducted by the Massachusetts Division of Marine Fisheries (DMF) and program partners. Standard Operating Procedures (SOP) are provided for routine measurements and multi-project applications for water temperature and chemistry loggers, and for diadromous fish habitat assessments. The document serves two primary purposes: (1) to provide standardized and consistent sampling protocols to improve the traceability and reliability of water quality data; and (2) to guide sampling efforts to produce data that is acceptable and contributes to Waterbody Assessments [Clean Water Act (CWA), Section 305 (b)] and aquatic resource protection and restoration efforts under CWA Section 303 (d) conducted by the Division of Water Management, Massachusetts Department of Environmental Protection (MassDEP).

This QAPP adopts the standardized approach recommended by the U.S. Environmental Protection Agency (US EPA) and MassDEP and described in Godrey et al. (2001). This approach contains 24 elements necessary to construct a successful and consistent QAPP. The Introduction contains the 24 elements and follows the formatting and terminology described in Godfrey et al. (2001) and MassDEP (2005). The remainder of the QAPP is comprised of five SOPs that provide specific direction for water quality monitoring related to Water Temperature Loggers (SOP 1.0), YSI 6-Series Probe Sondes, (SOP 2.0), Rainbow Smelt Spawning Habitat Assessment (SOP 3.0), River Herring Spawning and Nursery Habitat Assessment (SOP 4.0), and Fish Kill Investigations (SOP 5.0). The 24 elements in the Introduction provide common and consistent structure to data collections and management.

1. QAPP Version.

The present QAPP version 2.0 is an update of the previous version 1.0 that was approved by MassDEP in 2009 (Chase 2010) and applied by DMF during 2008-2018. Minimal changes were made to SOPs 1-3 for this update. The primary changes to QAPP 2.0 are the additions of an SOP on fish kill investigations, guidance on use of YSI 556 water chemistry instrument, and more detailed updates to SOP 4.0 on river herring spawning and nursery habitat assessment. Future QAPP revisions will depend on changes to methodologies and applications, and program objectives.

2. Equipment Disclaimer

References to commercial products and manufacturers do not indicate the endorsement of any products or companies by DMF or program partners. It is necessary to specifically name each piece of equipment so that QA/QC protocols can be developed around each product's specifications. SOP 2.0 on water chemistry loggers is directed to the use of Yellow Springs Incorporated (YSI) water quality sondes because all participating DMF projects use this equipment. However, the 24 elements of the Introduction are not dependent on specific sampling instruments. The SOPs can be readily modified by program participants in appendices to match the equipment specifications of items not listed in this version to water quality criteria.

3. Distribution List

DMF Project Staff:

Program Manager:	Greg Skomal, Ph.D.
Monitoring Coordinator:	Bradford Chase
Project QA/QC Analyst:	Bradford Chase
Project Field Coordinator:	Ben Gahagan
Project Field Coordinator:	John Sheppard
Project Field Coordinator:	Sara Turner, Ph.D.
Project Database Manager:	Sara Turner, Ph.D.

MassDEP Reviewers:

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Program Participants:

The following agency staff may utilize one or more of the SOPs under this QAPP. Additional staff and seasonal employees may be added following training.

MassWildlife	Caleb Slater, Ph.D.
MassWildlife	Steve Hurley
DMF	Ben Gahagan
DMF	Sara Turner, Ph.D.
DMF	John Sheppard

4. Project Organization

DMF project staff that will administer this document are within the Recreational and Diadromous Fisheries Program. It is anticipated that future applications may include participants from other DMF programs, Department of Fish and Game staff, and partner organizations.

5. Program Background

DMF is responsible for managing diadromous fish resources in the coastal waters of Massachusetts and shares this responsibility with the Division of Fisheries and Wildlife in inland waters. There is long history of DMF recording water quality data to accompany fisheries monitoring and research projects. Data management for past projects has been done on a project-specific basis potentially reducing the comparability of the data for intra- and inter-agency uses. Improved electronic logger technology during the last two decades allows the attainment of high accuracy and precision during water chemistry sampling if consistent QA/QC procedures are applied. The projects outlined in this QAPP and future efforts will benefit from the application of standardized water quality sampling and data processing protocols. In addition, MassDEP's Watershed Assessments are a powerful tool to identify and initiate remediation for water quality problems that influence the health of aquatic life. A MassDEP-approved QAPP will allow DMF data to contribute to the Watershed Assessment process.

The effort to adopt standard protocols originated from DMF monitoring of rainbow smelt spawning habitat (Chase 2010), and related efforts to linking water quality and watershed influences to rainbow smelt spawning habitat. These field exercises and the increasing utility of the electronic loggers for aquatic habitat monitoring prompted the interest in standardized sampling and QAPP protocols. Interest also came from the NOAA grant partnership between the states of Massachusetts, New Hampshire and Maine tasked with developing a conservation plan for rainbow smelt (Enterline et al 2012). Staff from the three states operated under the QAPP Version 1.0 during 2008-2012 for smelt habitat assessment sampling.

An underlying goal of the first QAPP was to record information related to diadromous fish populations and habitat that could be integrated to DEP's assessment process for surface waters in Massachusetts. An important step was made towards this goal with the publication of DEP's CALM (Consolidated Assessment and Listing Methodology) Guidance Manual in 2018 (MassDEP 2018). The CALM manual describes the methods used to meet CWA reporting requirements under sections 305 (b) and 303 (d).
For the first time, the 2018 manual included Diadromous Fish Habitat assessment guidance that was based on information provided by SOP 4.0 river herring spawning and nursery habitat assessments, the DMF Diadromous Fish Restoration Priority List and DMF fish passage surveys (Reback et al. 2004-2005).

Project Organization

Program Manager: Greg Skomal, Ph.D., DMF. Reviews and approves all project proposals and project reports using the QAPP.
Monitoring Coordinator: Bradford Chase, DMF. Developed the first four SOPs and will continue to provide oversight for projects and share regional instrument calibration and maintenance tasks with Field Coordinators. Will evaluate field, laboratory and data management activities and maintain communications with the Field Coordinator and Database Manager.
QA/QC Analyst: Bradford Chase, DMF. Will review QAPP data and assign data status criteria. Will train other DMF staff to serve as future QA/QC analysts for specific projects.
Field Coordinator: Ben Gahagan, DMF. Will coordinate the deployment of temperature loggers and YSI sondes on the North Shore; and lead regional instrument calibration and maintenance responsibilities. Will be responsible for data processing for Onset temperature loggers; and will be trained to collect data for all SOPs.
Field Coordinators: Sara Turner, Ph.D., and John Sheppard, DMF. Will coordinate the deployment of temperature loggers and YSI sondes for Southeastern Massachusetts. Will share regional instrument calibration and maintenance

responsibilities with the Monitoring Coordinator. Will be responsible for data processing for Onset temperature loggers; and will be trained to collect data for all SOPs. John Sheppard developed the Fish Kill SOP and will be the primary contact for diadromous fish kills.
Database Manager: Sara Turner, Ph.D., DMF. Will maintain databases for the QAPP and be trained to collect data for all SOPs. Will be responsible for processing data from YSI sondes.

6. Program Objectives

The primary program objective is to develop standardized data collection and processing protocols for water quality monitoring related to specific diadromous fish monitoring projects. The following SOPs will serve ongoing projects that were developed with the objective of producing comparable and reliable water quality data.

- SOP 1.0: Water Temperature Logger
- SOP 2.0: YSI 6-Series Multi-Probe Sondes
- SOP 3.0: Rainbow Smelt Spawning Habitat Assessment
- SOP 4.0: River Herring Spawning and Nursery Habitat Assessment
- SOP 5.0: Fish Kill Investigations

The first two SOPs have received wider use for among other DMF projects. Minimal changes were made to SOP 1-2 and to SOP 3.0, which is used only for one specific DMF project. QAPP update. SOP 4.0 has provided a tool for assisting the assessment and prioritization of diadromous fish restoration projects. SOP 5.0 is a new addition to Version 2.0 that allows improved standardization and documentation of DMF's field investigations of fish kill events. More specific details on project objectives and the target watersheds are presented in the individual SOPs. An important secondary objective is to provide data that can contribute to MassDEP's programmatic objectives of assessing the ability of water bodies to support designated uses (CWA, Section 305(b)) and remediating pollutant loads (CWA, Section 303(d) under their Watershed Assessment process (MassDEP 2005).

7. Data Quality Objectives

Parameter-specific data quality objectives are provided in each SOP and summarized in Table 1.1. The QAPP's basis for data quality control and assurance will be criteria established for the data quality indicators of precision, accuracy, completeness, comparability and representativeness (MassDEP 2005).

Precision. Precision is a measure of the proportion of agreement among replicate measurements. For most SOP parameters, precision will be sampled and evaluated by criteria established for the relative percent difference (RPD) of duplicate samples. Acceptable RPDs will be typically ≤5% for laboratory and multi-probe water chemistry sonde measurements and ≤35% for nutrient and productivity measurements.

Accuracy. Accuracy is the degree to which a recorded measurement varies from a true or expected value. Accuracy for multi-probe water chemistry sonde measurements will be assessed comparing pre-deployment and post-deployment calibration results to specifications established for standard solutions. Accuracy for temperature sensors will be checked against NIST-certified thermometers (National Institute of Standards and Technology). Where appropriate, the SOPs will outline the use of laboratory and trip blanks to contribute to assessments of accuracy. Accuracy warning and control limits for parameters will be established using standard deviation criteria on the departure from seasonal and station means.

Representativeness. Data representativeness refers to the extent to which measurements represent the true environmental or physical condition. This attribute is addressed through site selection criteria in "Station Selection" sections for each SOP. For example, rainbow smelt spawning habitat stations are selected from a state-wide list of known smelt spawning riffles where river flow is well-mixed and does not routinely receive saline water from the salt wedge.

Completeness. Data completeness refers to the amount of valid data collected as a proportion to the targeted sampling frequency. Weather, instrument failure and other conditions can result in incomplete or failed measurements in the course of a sampling season. The range of acceptable completeness for targeted measurements will be 75-100% for all SOPs.

Comparability. Data comparability refers to the extent to which data from one study are comparable to data collected for similar parameters during previous studies or from other areas. The documentation of sampling methods, data processing and QA/QC reviews will be used to determine data comparability over time. It is an important objective of the QAPP to improve and document data comparability for future surveys and resource management decisions.

8. Training Requirements

Program participants must become familiar with all aspects of the QAPP, SOPs and instrument manuals that guide sampling. Training sessions will be conducted by the Monitoring Coordinator under each SOP for program participants. Applications of SOP 4.0 can include unsupervised volunteer efforts. In these cases, the Monitoring Coordinator will conduct training sessions and initial trips with SOP 4.0 partners. It is expected that most SOP 4.0 trips will be lead by agency staff trained as program participants. Field Coordinators will be trained to collect field data for all SOPs and prepared to conduct training for program participants.

9. Documentation and Records

Standardized field and laboratory calibration forms will be used for all data collection covered within the QAPP. Templates of data forms are provided in each SOP and electronic templates are available upon request from the QAPP monitoring coordinator, brad.chase@mass.gov.

Field forms are constructed from Excel spreadsheets and are relational to spreadsheets where data will be entered and stored. Program participants will be trained to use field forms, calibration forms and enter data to spreadsheets. The Program Field Coordinator and Database Manager will process field and calibration data, and the program QA/QC Analyst will review and classify datafiles. Following review and final data classification, annual datafiles will be saved as read-only files in a shared server folder (DMF W:\ drive) that all participants can access. Back-up annual files will be saved the DMF P:\ drive by the Database Manager and QA/QC Analyst. Sampling stations will be documented with photographs and by recording the GPS location. The station documentation will be stored in an adjoining shared server folder.

10. Sampling Process

Sampling Safety. Sampling under SOPs 1.0-3.0 can occur in coastal rivers during spring. When river flows are elevated these conditions can be challenging, dangerous, and may compromise sampling methods. Field coordinators should monitor precipitation forecasts, stream flow gauge stations (when available) and use their best professional judgment (BPJ) when making decisions on river deployments. Field trips should be made with at least two staff. Exceptions can occur in small streams, primarily under SOP 1.0. Field staff should notify their supervisors of their plans before each field trip. Waders should be used for most in-stream work, although hip boots or knee boots are suitable for smaller streams and during the summer's low flows. Waders should be worn with a chest belt to reduce inadvertent water intrusion. SOP 4.0 will be conducted from small boats in most cases. Staff should wear Coast Guard-approved life vests during boat trips. All boat deployments should be accompanied with an extra life vest, paddle, anchor, and cell phone or VHF radio.

Design Considerations. Water quality sampling under this program targets specific river and lake locations used by diadromous fish for spawning, nursery and migratory habitat. Therefore, the approach to monitoring is to select fixed stations that can be monitored by DMF during critical life-stage periods on an annual basis. Probability based designs which are most suitable for watershed basin or state-wide water quality assessments (MassDEP 2005; and DeSimone et al. 2001) were judged impractical for retrieving detailed information on specific habitats used by individual diadromous fish runs.

The SOPs 1.0 and 2.0 serve more as instrument operation and maintenance plans for the project-based monitoring that occurs under SOPs 3.0 - 5.0. SOP 3.0 provided guidelines for delineating smelt spawning habitat and monitoring biotic and abiotic characteristics of the spawning habitat. SOP 4.0 provided guidelines for river herring spawning and nursery habitats, primarily at lakes and ponds. It is expected that future DMF applications for SOP 1.0 and 2.0 will be developed independently of the smelt and river herring monitoring projects. In all cases, water quality data processing and quality assurance will be guided by common procedures.

11. Sampling Method Requirements

Sampling methods for each program project are described in the corresponding SOP sections.

12. Sample Handling & Custody Requirements

Laboratory analyses for SOP Sections 1.0 and 2.0 are limited to instrument calibration. Calibration procedures are outlined in each SOP. SOP 4.0 involves field collection and laboratory analysis of surface water nutrient samples and SOP 5.0 may include water sample collection for laboratory analysis. These handling procedures will be described, and a chain of custody form will be supplied in SOP 4.0 and 5.0. For all field and laboratory processes, the date and names of the sampling crew and instrument identification must be recorded on data forms. Sample labeling and numbering will be synchronized among the program participants. Both water quality and biological data samples will be assigned an alphanumeric label that denotes, in order: location (text, 2-3 letters), year (2 numbers), sample type (text, 1-2 letters), annual sample number (1…x). For example, the first total phosphorus sample collected in the Parker River, during 2020 would appear as: PR20-TP1.

13. Analytical Methods Requirements

The reporting of laboratory analytical methods only applies to SOP 4.0 and 5.0. The analytical methods, holding times and parameter specifications for the analytical laboratory are described with citations in these SOPs.

14. Quality Control Procedures

Quality control procedures will be outlined in each SOP section. The following three main components of quality control will be applied in each SOP where applicable: pre- and post-deployment instrument calibrations with accuracy and precision checks, analysis on the similarity of replicates, and outlier review using specified flags related to deviations from seasonal and station mean data. For projects where different crews are applying the same methods, an annual QA/QC meeting should be held to discuss sampling methods and review quality control results. At these meetings, side-by-side measurements of the same model instruments can be made if questions are raised over sensor results. The comparability of side-by-side measurements can help isolate quality control problems.

15. Instrumentation/Equipment Inspection and Testing

Instrument testing and maintenance will be outlined in each SOP section and recorded on specified forms during each calibration. Laboratory balances used for supporting wet and dry chemistry applications are inspected and calibrated annually at DMF’s Annisquam River Marine Fisheries Station and New Bedford bacteriological laboratory by a certified vendor, with test documents maintained on file at the laboratories.

16. Instrumentation Calibration & Frequency

Instrument calibration information is outlined in each SOP section. With a few exceptions, the YSI water chemistry sonde will be the only instrument calibrated for QAPP applications. The YSI sonde calibration procedures are provided in SOP 2.0. Specific project applications for water chemistry sondes are outlined in SOPs 3.0 - 5.0.

17. Inspection of Supplies

Field and laboratory data forms will be consistently used by all program participants. The data forms will be inspected at the start of each field season for completeness and applicability. Program participants will inspect all calibration solution standards to ensure they have not expired. Expired standards may be used for calibrations for up to six months after the expiration date, after which they can only be used as a pre-calibration wash solution. Coolers and other carrying containers for field instruments and samples will be thoroughly cleaned at the start or end of each week during the sampling season.

18. Data Acquisition Requirements

Annual requests will be made to the US Geological Survey (USGS) for discharge data from sampled rivers with stream flow gage stations. Secondly, annual requests will be made to the National Climatic Data Center (NCDC) for air temperature and precipitation data from weather stations near river sampling stations. In both cases, real-time values of some parameters are available on the agency's web sites. The real-time data should be considered preliminary for this QAPP. USGS and NCDC data undergo a QA/QC review and classification process. This could result in changes to real-time data. It is presently more efficient and prudent to wait until the sampling season is over and retrieve all data needs for the calendar year following agency classification. These data will be processed and included in water chemistry datafiles as daily observations for each sampling season.

19. Data Management

All laboratory calibrations and field data collections will be recorded on approved forms listed below. All form templates are stored in the DMF shared computer drives (W:\) under the Diadromous folder. Field and calibration forms should be filled out on the day that project activity occurred and stored in individual hard copy files by the program participant. All forms should be inspected for completeness, initialed and dated by the project participant. At the end of a sampling season, all forms for a given project should be inspected by the Field Coordinator or QA/QC Analyst to flag errors or missing fields. Any identified problems should be discussed with field staff and corrections should be documented. Following this activity, the data will be ready for entry into Excel datasheets.

Following data entry, the QA/QC Analyst or Database Manager (if the Database Manager did not enter data) will audit the Excel datafiles by visual comparison with field forms. The audit will cover 100% of entered data. Discovered errors will be corrected and a tally of field sheet, keypunch, and other errors will be recorded in a QA/QC review worksheet in each annual datafile. Once the audit is complete, the auditor will indicate the QA/QC status on the datafile and enter their name and the month/year.

The QA/QC Analyst should review the data and classify the QA/QC and data status using the classes listed below. The QA/QC status refers to the review stage for the entire datafile. When all QA is finished the QA/QC Analyst will mark the QA status box as *Complete* and enter the month and year. The data status classes refer to the status of data when the QA review is completed. Data will be stored in the DMF shared computer drives (W:\) with back-ups in the personal (P:\) drives of the Database Manager or QA/QC Analyst. Once a datafile has been validated by the QA/QC Analyst it will be saved as a read-only file in the W:\ drive and backed up in the P:\ drive.

QA/QC Status

1. Draft. Data processing is in progress, and QA/QC has not been conducted.

2. Preliminary. Data processing is complete, but QA/QC is not complete. Data can be used for internal project summaries.

3. Complete. All data processing and QA/QC review is completed.

Data Status

1. Preliminary. Data have been entered from field sheets or downloaded but QA/QC review is not complete.

2. Censored. Data are eliminated because of instrument failure or QA/QC performance.

3. Conditional. Data are fully audited, and QA is complete, but have deficiencies that are documented and may limit use.

4. Final. Data are fully audited, checked and acceptable.

Table 1.1. Data quality objectives of accuracy and precision for QAPP water chemistry parameters.

PARAMETER	UNITS	ACCURACY CRITERIA (±)	PRECISION CRITERIA (RPD)
Temperature	ºC	0.3	5%
pH	Standard units	0.2	5%
Dissolved Oxygen	mg/l	0.5 or 5% of standard*	5%
Dissolved Oxygen	% saturation	5% of standard	5%
Specific conductance	mS/cm	2% of standard	5%
Salinity	ppt (derived)	0.5 or 2% of standard*	5%
Turbidity	NTU	2.0 or 5% of standard*	25%
Total Nitrogen	mg/L	85-115% recovery of lab. fortified sample matrix	35% (field) 15% (laboratory)
Total Phosphorus	ug/L	85-115% recovery of lab. fortified sample matrix	35% (field) 15% (laboratory)

Table 1.2. List of data forms used in Standard Operating Procedures 1.0 – 5.0.

Form 1.1	Water Temp. Logger	1.0	Temp. logger deployment and QA
Form 2.1	YSI Sonde Calibration	2.0	YSI sonde calibration: long-term
Form 2.2	YSI Sonde Calibration Review	2.0	Compare calibration to SOP specifications
Form 2.3	YSI Sonde QA/QC	2.0	QA/QC review and data status
Form 2.4	YSI Sonde Calibration	2.0	YSI sonde calibration: grab samples
Form 3.1	Field Water Chemistry and Flow	3.0	Periphyton field station water data
Form 3.2	Nutrient Data	3.0	Periphyton field station nutrient data
Form 4.1	River Herring Habitat Assessment	4.0	Documentation of field habitat assessments
Form 5.1	Fish Kill Investigation	5.0	Field form to document fish kill events
Form 5.2	Fish Kill Sub-Sample	5.0	Field form for fish kill biological data

Censored data cells will be marked with a red color code in the Preliminary datasheets and empty in Final Worksheets. *Conditional* data cells will be marked with a yellow color code in both Preliminary and Final datasheets.

20. Assessment and Response Actions

The evaluation of field, laboratory and data management activities for all SOP sections will be overseen by the QA/QC Analyst and will involve in-season and post-season communication with program participants and a series of validation checks during data collection and processing. The DMF field activities for SOPs 1.0-3.0 will involve only a few trained staff. Communication among project staff will be a routine process to ensure project protocols and objectives are met. Raw data form checks and datafile audits are important validation steps that will identify minor errors and result in corrective action for systematic errors. The datafile audit will include a tally of errors by type and data entry staff. A meeting will be held at the conclusion of each annual project audit to discuss data quality and identify recurring errors. We have experienced consistently low frequency of data entry error of typically <3% (No. of errors per 100 keypunched cells) for water quality datafiles. Error rates above 3% will prompt specific discussions on corrective actions. All QA/QC decisions and corrections will be recorded in the QA/QC Review worksheet adjoined to each annual datafile.

21. Reports

Reports will be written for each specific project conducted under SOP 3.0 and 4.0 and include a discussion on QA/QC. Data collected under SOP 1.0 and 2.0 will be used in the project reports on smelt and river herring population and habitat assessments. In addition, all validated datafiles will be shared with program partners and available as public property to any interested party. The schedule and detail of written summary reports will depend on the objective of each SOP and in response to varied requests to meet DMF management needs, to assist environmental permit review, to meet environmental permit enforcement requests and other external requests. Deviations from the QAPP and SOPs will be documented in the project reports.

22. Data Review

Common methods and terminology will be used for documenting the QA/QC review and data status in each SOP Section of this QAPP (see No. 19. Data Management).

23. Validation and Verification Procedures & Requirements

Specific processes for validating data are provided in each SOP, including parameter specific validation criteria tables. Following entry of field data into the corresponding datafiles, each annual datafile will be reviewed by the following four steps (where applicable):

1. **Data Audit**. Data are compared to field sheets (100% visual audit for keypunched data) to identify entry errors, remove pre- and post-deployment data and flag potential outliers.

2. **Calibration Review.** For YSI sondes and a few other applications, the pre- and post-deployment calibration data will be evaluated following specific performance criteria on accuracy related to standard solutions and the instrument manufacturer's specifications.

3. **Replicate Analysis.** The similarity of replicates will be reviewed in relation to performance criteria on sampling precision.

4. **Outlier Review.** Outliers flagged during auditing shall be graphed and compared to deviations from the parameter means and medians for both seasonal and station data series. Outliers that exceed warning (±2 SD) and control (±3 SD) limits will be subject to responses outlined in each SOP.

24. Reconciliation with Data Quality Objectives

The final status of sampled data will depend on the data classification criteria described in Data Management (see No. 19 Data Management). Decisions on data classification are dependent on the calibration performance of each sensor as assessed by accuracy and precision tests. For the water quality data, corrective actions will be made on units of data from individual deployments (or between calibrations). Within deployments, classifications and corrective actions will be specific for each parameter. For example, it will be possible for an entire season of specific conductivity data to be classified as *Final* while the DO data in the same file is classified as *Final, Conditional* or *Censored* for different deployments in the same datafile. Parameter specifications and validation criteria are provided in each SOP along with direction for corrective actions and guidance for data classification.

ACKNOWLEDGEMENTS

The first version of this QAPP received extensive review from numerous staff in the Department of Fish and Game, MassDEP and other Commonwealth of Massachusetts agencies. We sincerely appreciate this assistance to develop the 2010 QAPP. Version 2.0 of the QAPP represents more of an update of Version 1.0 without major

changes to monitoring practices and applications. Version 2.0 was mainly reviewed by DMF staff, with significant contributions from the QAPP co-authors and former DMF staff, Mike Bednarski. We are also grateful to the efforts of MassDEP staff who both reviewed the QAPP and worked with us to better integrate our data collections, formatting, and reporting to ongoing MassDEP processes related to Wetlands Protection Act processes for protecting aquatic resources in Massachusetts.

NOTES AND UPDATES

Optional Methods. Included in the methods sections for each SOP are *options* for field methods, QA/QC, and data processing. The options are suggestions for different approaches to an operating procedure or to troubleshoot problems. In all cases, the options are not requirements of this QAPP and the required procedures should be first attempted and documented. It is expected that technologies and methodologies for using these electronic instruments will be periodically updated. These changes will result in modifications to some current standard operating procedures. The application and conceptualization of optional methods has assisted the update from QAPP Version 1.0 to 2.0 and may help identify better approaches for water quality monitoring for future applications of this QAPP.

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ACRONYMS

AFDW	Ash Free Dry Weight
BPJ	best professional judgment
cfs	cubic feet per second
CALM	Consolidated Assessment and Listing Methodology (MassDEP)
CWA	Clean Water Act
DAR	Massachusetts Department of Agricultural Resources
DDW	deionized-distilled water
DMF	Massachusetts Division of Marine Fisheries
DEP	Department of Environmental Protection
DFG	Massachusetts Department of Fish and Game
DO	dissolved oxygen
EOEEA	Massachusetts Executive Office of Energy and Environmental Affairs
GPS	Global Positioning System
MA	Massachusetts
MassDEP	Massachusetts. Department of Environmental Protection
	BRP MassDEP Bureau of Resource Protection
	BWSC (ER) MassDEP Bureau of Waste Site Cleanup (Emergency Response)
	DWM MassDEP Division of Watershed Management
	ESF MassDEP Environmental Strike Force
MassWildlife	Massachusetts Division of Fish and Wildlife
ME	Maine
MGL	Massachusetts General Laws
NCDC	National Climatic Data Center
NH	New Hampshire
NIST	National Institute of Standards and Technology
NMFS	National Marine Fisheries Service
NOAA	National Oceanic and Atmospheric Administration.
NTU	Nephelometric Turbidity Units
QAPP	quality assurance program plan
QA/QC	quality assurance/quality control
RPD	relative percent difference
RSD	relative standard deviation
SD	standard deviation
SOP	standard operating procedures
SWQS	surface water quality standards (*Mass*DEP)
TN	total nitrogen
TP	total phosphorus
UNH	University of New Hampshire
US EPA	United States Environmental Protection Agency
USGS	United States Geological Survey
USFWS	United States Fish and Wildlife Service
YSI	Yellow Springs Incorporated

CONVERSION FACTORS

Multiply U.S. Customary Units	By	To Obtain Metric Units
inch (in.)	2.54	centimeters (cm)
foot (ft.)	0.3048	meters (m)
mile (mi)	1.609	kilometers (km)
square miles (mi^2)	2.590	square kilometers (km^2)
acre (A)	0.004047	square kilometers (km^2)
acre (A)	0.404686	hectares (ha)
cubic feet (ft^3)	0.02832	cubic meters (m^3)
Gallons (gal)	3.785	liters (L)
Temperature Conversion		
Celsius degrees (°C)	1.80*(°C) +32	Fahrenheit degrees (°F)
Fahrenheit degrees (°F)	0.5556*(°F-32)	Celsius degrees (°C)

EQUATIONS

Relative Percent Difference (RPD)

A measure of precision for duplicate samples (X_1 and X_2)

$$RPD = [\ (X_1 - X_2)/\ ((X_1 + X_2)/2)\ ] * 100$$

Relative Standard Deviation (RSD)

A measure of precision for three or more replicates (X_1, X_2, and X_3)

$$RSD = [\ SD/\ ((X_1 + X_2 + X_3)/3)\ ] * 100$$

Standard Operating Procedures

Section 1.0 Water Temperature Loggers

SCOPE AND APPLICATION

Monitoring Objective. Metabolic and reproductive processes in ectothermic fish have evolved in response to natural temperature patterns. Natural and anthropogenic disruptions to water temperature can have acute and chronic consequences to individual fish and fish populations. Water temperature is also an important environmental cue for different stages of river herring life history (Loesch 1987; Greene et al. 2009). Electronic data loggers are deployed to record high quality, continuous water temperature data. The water temperature data will provide seasonal and annual trends that can be related to diadromous fish life history and habitat requirements.

Data Quality Objective. The accuracy of data loggers must be ± 0.3 ºC of the true temperature value and must be confirmed with the accuracy checks described below. The precision of the data loggers must be at least 95% (<5% relative percent difference, RPD) as determined from duplicate recordings at the same time and space.

INSTRUMENTS

A variety of instruments are available to accurately record continuous water temperature for river and marine deployments. Loggers with a listed accuracy of ±0.3 ºC over the range of 0 to 30 ºC and battery capacity to conduct annual deployments can be approved for QAPP deployments. Loggers that exceed this accuracy threshold are not preferred. Present project applications are using the Onset Water Temp Pro (#U22-001) for all deployments. This logger has a resolution of 0.02 °C and accuracy of 0.2 °C over a range of 0 to 50 °C.

DEPLOYMENT

Pre-Deployment Procedures

Time Check. Compare instrument time and date to cell phone time and date. Adjust if needed using the logger's launch/readout software.

Battery Check. Record battery strength; annual deployments should not have less than 90% capacity, and shorter term, or back-up deployments should not have less than 80%.

Accuracy Check. Two acceptable methods are available to check logger accuracy. The preferred method is to compare the logger to a thermometer traceable to National Institute of Standards and Technology (NIST) standards and accurate to ±0.2 ºC. Fill a bucket with water and allow the bucket to sit ≥1 hour at constant room temperature (20 °C ±5 °C). Record logger and the certified thermometer temperatures at the same bucket depth. The logger tested will be acceptable if its measurement is within ± 0.3 ºC of the NIST-traceable thermometer.

If a certified thermometer is not available, use a bucket of crushed ice with distilled water to check logger accuracy. Allow the ice and water to acclimate for 20 minutes and immerse logger for five minutes. The logger is acceptable if the measurement is 0 ºC ±0.3 ºC. Loggers that fail these tests should be tested again and not be deployed following two failures. If accuracy check results cannot be reviewed from the logger datafile until logger retrieval, then evaluate both the pre-and post-deployment accuracy checks during the post-deployment review.

Precision Check. Test the logger precision by recording duplicate temperature measurements separated by two minutes at the same time as the bucket accuracy check. Calculate the RPD of the two samples. *Back-up Logger Option:* when deploying a back-up logger, simultaneous measurements from each logger placed side-by-side in the water bath can be used to check precision and comparability among loggers. Loggers with RPD ≤5% and acceptable accuracy are suitable for deployment. Loggers that exceed this level of precision should be tested again in the water bath at a high and low temperature in order to isolate possible causes for lower precision. Loggers with acceptable accuracy and precision at both temperatures are suitable for deployment as primary loggers. Loggers with lower (but acceptable) accuracy and lower battery strength can be reserved as back-up loggers.

Ryan TempMentor. Ryan TempMentor loggers were approved for Version 1.0 but have since been discontinued. Data series that previously used Ryan TempMentor loggers should document the change in instruments.

<u>Deployment Procedures</u>

Location Selection. Pick river locations that have noticeable landmarks and shelter from full sunlight and visibility. The flow at the site should have good mixing and provide at least 0.2 m of depth over the logger at all times. Record GPS coordinates and landmarks. Loggers used in diadromous fish runs should be deployed upstream of the salt wedge (determined by existing salinity data, or the presence of barnacles and shellfish) in order to record freshwater temperature. Marine locations accessed by scuba diving should be associated with visible underwater landmarks and not be subject to disruption from fishing gear or other divers.

Schedule. For most diadromous fish projects, the loggers will be deployed annually. Mid-season checks are recommended but not required given the lack of problems experienced under this SOP with annual deployments and the added process to service all locations. When applicable, mid-season checks can be conducted to check the logger battery, accuracy, precision and to be sure it is covered by water during low-flow periods. The minimum deployment period for diadromous fish spawning runs is March 1st –June 30th. For this period loggers should go out by the end of February. Marine loggers are deployed and retrieved on one date per year. Therefore, scheduling can be flexible as long as battery life is considered. Scheduling scuba visits during warmer months is usually preferred.

Logger Set-up. Activate loggers at hourly intervals on-the-hour. Conduct water bucket checks for accuracy and precision. Back-up loggers should be activated to begin logging at the same time and date as the primary loggers.

Logger housing/weights. The logger housing/ weights used to hold substrate position should be suitably heavy for all possible conditions and have low relief to avoid shifting and low visibility to avoid attracting attention. We have had routine success using a 1 ft section of 1in rebar. The logger should be secured to the rebar with tie-wraps and electrical tape. The logger and the outer tape should both be labeled with agency name, project contact name and phone number.

Record Keeping. Record logger serial number, deployment history, location, and all other fields listed on Water Temperature Logger Deployment Form 1.1 prior to deployment.

<u>Recovery Procedures</u>

Schedule. Loggers used for diadromous fish projects should be recovered during the lower flows of July-October. Most river and marine stations will be designated for annual coverage and require deployment of a new logger on the recovery date. For cases of short-term deployments at spring spawning runs, the loggers can be retrieved after June 30th.

<u>Post-Deployment Procedures</u>

Quality Assurance. After recovering loggers, users should repeat the “Pre-deployment Procedures”. Loggers that fail accuracy tests should be tested again. Data from loggers that had acceptable pre-deployment quality control checks yet failed two post-deployment accuracy tests (and the difference from NIST-traceable thermometer is <1.0 ºC) should be classified as *Conditional*, and the loggers should be returned to manufacturer for service before deploying again. Data from loggers that fail two post-calibration checks by >1.0 ºC should be *Censored*, as this level of error is considered unacceptable and associated with logger failure. Transfer logger data to an Excel datafile and confirm the check start and end times against Form 1.1 records. No additional audit of logger data is needed for loggers with acceptable pre- and post-deployment quality control checks. All remaining fields of Form 1.1 should be filled.

Calculation of Daily Mean Temperature. Loggers should be set to record on-the-hour at hourly intervals, allowing for 24 measurements to comprise the daily mean temperature. Daily mean water temperature will be calculated in the Excel spreadsheet from the raw data using the 24 daily measurements from midnight (00:00) to 23:00.

Data Calculations (*Option*)**.** Users can select to process daily mean temperature data into annual tables that calculate monthly mean, minimum and maximum temperature, and histograms that illustrate the number of days that the daily mean temperature occurred within 1 ºC bins. When information is available on thermal requirements for specific fish, data can be processed as daily maxima to compare to acute thermal criteria and weekly average maxima for comparisons to chronic criteria (Todd et al. 2008). Daily maximum temperature is the highest 2-hour average temperature during a 24-hour period. Weekly average maximum temperature is evaluated by comparing the 7-day means of daily mean temperatures to chronic thermal requirements for each fish species.

Logger Cleaning. Loggers should be soaked in soapy water to loosen dirt and attached marine life. Onset loggers deployed in freshwater require only moderate scrubbing with a scour pad, except the optical port should only be cleaned with a sponge. Marine deployments should always use port protective caps and cover the logger with electrical tape to avoid excessive marine growth.

Data Classification. Use the Excel temperature logger template to review raw data and quality control checks. The final Excel datafile should have four attached worksheets labeled: *Raw*, *Mean*, *Graph*, and *Form 1.1*. A time-series graph of the logger data is a good visual tool to flag obvious problems (ex. logger removal from water) and check deployment/retrieval times. The QA/QC Analyst should review the data and classify the QA/QC review status and data status using the classes listed below. The QA/QC status refers to the review stage for the entire datafile. When all QA/QC is finished the QA/QC Analyst will record QA/QC status as *Complete* and enter the month and year. The data status refers to the status of data when the QA/QC review is completed.

QA/QC Status

1. Draft. Data processing is in progress, and QA/QC has not been conducted.

2. Preliminary. Data processing is complete, but QA/QC is not complete. Data can be used for internal project summaries.

3. Complete. All data processing and QA/QC review is completed.

Data Status

1. Preliminary. Data have been entered from field sheets or downloaded but QA/QC review is not complete.

2. Censored. Data are eliminated because of instrument failure or QA/QC performance.

3. Conditional. Data are fully audited, and QA is complete, but have deficiencies that are documented and may limit use.

4. Final. Data are fully audited, checked and acceptable.

Data Storage. Each location will have an annual Excel datafile that is named by river and year (ex. Parker River-2005). All Excel approved datafiles should be stored as read-only files on the DMF shared drive W:\ with back-up files saved in the Database Managers' personal drive (P:\).

Back-up Loggers. Back-up loggers should be deployed at any stations with ≥10 years of records. The start times should be synchronized for the primary and back-up loggers. Back-up loggers should be subject to the same quality assurance as primary loggers, although the data and documentation can be included on the same logger Excel datafile by adding a *Back-up* worksheet. The primary purpose of the back-up logger is for data use in the event the primary logger is lost. However, the records of two deployed loggers can be compared by evaluating the percent agreement of daily mean temperatures. Given the high accuracy of these instruments, agreements near 100% are expected. *Option:* the user can elect to process data from the logger with the best performance during accuracy checks. This approach is acceptable if the loggers had a common time stamp and the RPD of daily mean temperatures is ≤5%.

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Form 1.1 Water temperature logger deployment and QA/QC form.

MASSACHUSETTS DIVISION OF MARINE FISHERIES

Water Temperature Logger Deployment and QA/QC. **Form 1.1**

Location:	*(place, Town)*	**GPS Position:**	*(Latitude/Longitude)*
		Logger:	*(model/serial number)*
Start:	*(date, time)*	**Instrument Accuracy:**	*(± ºC at specified range)*
Deployment:	*(date, time)*	**Deployment History:**	*(purchase date and No.)*
Recovery:	*(date, time)*	**QA/QC Analyst:**	*(name)*
		QA Status:	(*Draft, Preliminary, Complete*)
		Data Status:	(*Preliminary, Censured, Conditional, Final*)

Pre-Deployment Check

			ACCURACY			*PRECISION*			
Date	**Time**	**Logger** (SE#)	**Logger** (ºC)	**Cert. Therm.** (ºC)	**Deviation** (ºC)	**RPD (1)**	**RPD (2)**	**RPD (%)**	**Notes**

Primary Logger Battery: *(% and/or V)* **Acceptable accuracy deviation:** ≤ 0.3 °(

Back-up Logger Battery: *(model/serial no. and % and/or V)* **Acceptable precision RPD:** ≤ 5.0 %

Internal Clock: *(check with cellphone and note adjustment or mark "correct")*

Post-Deployment Check

			ACCURACY			*PRECISION*			
Date	**Time**	**Logger** (SE#)	**Logger** (ºC)	**Cert. Therm.** (ºC)	**Deviation** (ºC)	**RPD (1)**	**RPD (2)**	**RPD (%)**	**Notes**

Primary Logger Battery: *(% and/or V)* **Internal Clock:** *(note adjustment or mark "correct")*

Back-up Logger Battery: *(% and/or V)* **Daylight Savings Time:** *(note if adjusted for DST)*

Record of Deployment Start: *(compare time data to deployment notes)*

Record of Deployment End: *(compare time data to deployment notes)*

Back-up Comparison: *(comment on timing agreement and certified thermometer check)*

Back-up Daily Mean: *(comment on % agreement)*

Notes:

SCOPE AND APPLICATION

Section 2.0 of the QAPP's Standard Operating Procedures (SOP) is intended to standardize instrument handling, calibration, deployment, post-deployment procedures and maintenance for multi-sensor water chemistry sondes. Standardized protocols are necessary to improve the traceability and reliability of the data. These procedures are required for all deployments of YSI 6-Series (6920, 6820, and 6600) sondes. Separate protocols are provided for unattended logging and grab samples. The SOP was developed using YSI 6-Series Operations Manual (YSI 2006) and the 2005 YSI technical note, "Deployment and Data Quality Assurance", and over 10 years of experience using YSI products. The SOP should be revisited and updated periodically to account for changing technologies and improving application knowledge. This document does not cover all aspects of instrument calibration and operation. It is important that users of YSI 6-Series sondes become familiar with the YSI operations manual and follow the manual's instructions. The SOP protocols offer additional points of clarification on YSI manual instructions and quality control and assurance procedures specific to our applications monitoring diadromous fish habitat.

<u>Monitoring Objectives</u>. Electronic multi-probe sondes will be deployed to record both grab samples and continuous water chemistry data within coastal river watersheds monitored for diadromous fish resources. The recorded data will assist ongoing fisheries sampling programs and interagency efforts to manage aquatic resources. The SOP protocols can also be applied to DMF projects measuring water chemistry related to other aquatic marine resources and habitats.

SOP Update. The update of SOP 2.0 involves relatively minor improvements to protocols for using YSI 6-Series sondes. The program plan still relies on 6-Series sondes for most field projects. It is expected that new instruments will be adopted under QAPP V-2.0. In this event, appendices on the operations of new instruments should be added to the SOP. This SOP update also includes an appendix on the operational protocols for YSI's 556 MPS multi-probe water chemistry instrument.

INSTRUMENTS

This SOP was developed for YSI 6-Series multi-probe sondes that are presently used for diadromous fish and marine waters monitoring. The SOP update includes protocols for YSI 556 MPS in the Appendix. Other products are available that can meet the Program's data quality objectives. Program participants with other instruments can use the SOP's QA/QC guidelines while modifying the SOP with an appendix attachment to account for different sondes or sensors. The Monitoring Coordinator should maintain a current roster of instruments available for deployment for each program participant; and monitor the maintenance status and calibration performance of each instrument.

SPECIFICATIONS

Sensor resolution, range, and accuracy are provided by the manufacturer for each measured parameter Table 2.1. These specifications represent a baseline of expected performance and criteria for evaluating calibration results. It is our experience that properly calibrated and functioning sondes will provide results within these specifications, with few exceptions.

<u>Data Quality Objectives</u>. Water quality data within the accuracy range specified by YSI for each parameter probe should be attainable with accurate and consistent calibration. The acceptable SOP accuracy differs slightly from YSI specifications for temperature, dissolved oxygen (DO), and specific conductivity. It is our experience that the YSI accuracy listed for these parameters provides little margin for slight deviations. Therefore, we have adopted higher criteria for acceptable accuracy. These accuracy objectives can be monitored by conducting and reviewing pre-deployment and post-deployment calibrations. The precision of sensor parameter measurements is monitored in the laboratory during each calibration by recording the relative percent difference (RPD = (difference of two consecutive readings/ average of two consecutive readings) x100).

Table 2.1. YSI 6-Series sensor specifications and data quality objectives. An asterisk (*) in either ACCURACY column denotes "whichever is greatest" relative to the allowable deviation from the calibration standard.

PARAMETER (Units apply to columns 1-4)	RESOLUTION and RANGE	YSI ACCURACY (±)	SOP ACCURACY CRITERIA (±)	SOP PRECISION CRITERIA (RPD)
Temperature (ºC)	0.01 -5 to 45	0.15	0.3	5%
Depth (m)	0.001 0 to 61	0.12	0.12	5%
pH (standard units)	0.01 0 to 14	0.2	0.2	5%
DO (mg/l)	0.01 0 to 50	0.2 or 2% of standard*	0.5 or 5% of standard*	5%
DO (% saturation)	0.1 0 to 500	2% of standard	5% of standard	5%
Specific conductance (mS/cm)	0.001 0 to 100	0.5% of standard (+0.001 mS/cm)	2% of standard	5%
Salinity (ppt, derived)	0.01 0 to 70	0.1 or 1.0% of standard*	0.5 or 2% of standard*	5%
Turbidity (NTU)	0.1 0 to 1000	2.0 or 5% of standard*	2.0 or 5% of standard*	25%

Precision Check. Allow a bucket of tap water to acclimate to room temperature (≥1 hour). Place the sonde in the bucket and allow sensors to equilibrate to water temperature for at least 10 minutes. Once the sonde has equilibrated, record water chemistry on Form 2.1 and repeat measurements after two minutes. At this time, also check the temperature sensor against a National Institute of Standards and Technology (NIST) traceable thermometer.

LONG-TERM DEPLOYMENTS

Site Selection

Rainbow Smelt. Sondes should be deployed to record freshwater chemistry data in close proximity to smelt spawning habitat. The presence of the salt wedge should be avoided because it can confound the interpretation of the freshwater chemistry that influences adult fish attraction and egg survival. Ideally, a site above the influence of tide at an active spawning riffle should be selected. The site should have well-mixed flow and adequate depth to cover the sonde at all times and conceal the sonde from detection. Depths greater than 1 m should be avoided because retrieval can be difficult with high flows. Sites near the fresh and saltwater interface that experience the backing up of freshwater during high tide can be selected but will require enhanced data review to identify any salt wedge influence. Trial deployments can be made at such sites to confirm that tidal influence does not influence water chemistry. Avoid high pedestrian traffic locations where the risk of vandalism increases. Record location in latitude and longitude with GPS unit.

River Herring. See Section 4.0 on river herring spawning and nursery habitat.

Pre-Deployment Procedures

Calibration. Calibrations should be done in the laboratory at room temperature using a PC or laptop with EcoWatch software or with a YSI 650 MDS Display. The depth sensor is the one exception that can be calibrated in the field or laboratory. The calibration should occur within 24 hours of the deployment. Begin the calibration process with DO and continue with each parameter as described in the YSI manual. Record the calibration process on Calibration Form 2.1.

Calibration Rinses. Clean, unexpired calibration standards should be used for each calibration. Previously used standards can be used to rinse sensors, but not for calibration. Before each calibration step with a standard solution, the sensors should be rinsed once with deionized-distilled water (DDW) followed by a rinse with a previously used standard. A second rinse of DDW should be made prior to 0.0 NTU turbidity and specific conductivity calibrations. The sonde, with calibration cup off, should be shaken lightly prior to using the final standard to remove excess liquid from the sensors. The previously used standards should be discarded after one rinse.

DO Calibration

DO 6562 Sensor. Recent YSI 6-Series instruments use the optical DO sensor 6150. Several older instruments continue to use the rapid pulse DO 6562 sensor that has a replaceable Teflon membrane. Our long-term sonde deployments are routinely for 3-5 weeks. Because the DO membranes are unstable following installation, the membrane should be changed the day before or at least 6 hours before each deployment. If this is not possible, conduct a membrane "burn-in" in Discrete Run mode. Set the sonde to record DO at 4-second intervals in Discrete Mode. Allow sonde to run in this mode for 15 minutes to electrically stabilize membrane and sensor. After 15 minutes, confirm that the 4-second measurements have stabilized, and check DO charge and gain to confirm they are within the acceptable ranges (see *DO Troubleshooting*). Because the KCL electrolyte used with the membrane is corrosive to connectors and o-rings all sensors and sonde ports should be protected from the KCL electrolyte with paper towels.

Calibration Cup. Use YSI calibration cup with 1 inch of water. Do not submerge the DO membrane in water. Screw the calibration cup to the sonde for only 1-2 threads: air space is needed to vent with the atmosphere. Allow sonde to rest on its side for at least 10 minutes before calibrating.

Pre-Calibration Test. Turn on sonde in Discrete mode after sonde has acclimated with calibration cup. After 10 minutes, record % saturation (pre-calibration value for Calibration Form 2.1) and DO charge. Percent saturation should be near 100% and DO charge should be in range of 25-75. Proceed to calibrate if correct or to troubleshooting if not (see Technical Notes for an alternative DO calibration).

Calibrate DO. In the Advanced Menu set the Auto Sleep RS-232 option to "ON" for unattended logging and "OFF" for grab sampling. Conduct DO calibration in % saturation mode. If using a YSI 650 display or computer without barometer, enter barometric pressure (mm Hg) from laboratory barometer. If a barometer is not available, refer to YSI manual for using uncorrected barometer pressure measurements.

DO Optical Sensor 6150. The optical sensor has no membrane and requires less maintenance. The water-saturated air calibration for the 6150 sensor follows the same process as the 6562 sensor. YSI recommends that calibration error can be reduced by calibrating the 6150 sensor in saturated water using a bucket and air pump. For consistency, the QAPP Version 2.0 recommends continued use of the air calibration method. Refer to the YSI manual for optional calibration instructions and further technical comments on the optical DO sensor.

DO Troubleshooting (#6562). DO charge and gain readings are diagnostic tools for evaluating DO sensor performance. The gain of a properly calibrated DO probe should be in the range of -0.7 to +1.4. This can be checked under "Cal Constants" in the sonde's Advanced Menu if there is doubt over sensor performance or the acceptability of a calibration. DO charge should be in the range of 25-75. Values below this range can be caused by low concentration electrolyte or a tear in the membrane. Values above this range may be caused by anode oxidation or a failed sensor. If DO gain or charge is out of range, inspect the integrity of the membrane. Secondly, inspect the sensor anodes for oxidation. If the anode is tarnished or gray, recondition with YSI kit 6035. Thirdly, remove the DO sensor from sonde and check the DO charge: a reading of -0.8 to 1.2 indicates that the sonde is functioning correctly and the problem is likely a failed sensor.

DO Troubleshooting (#6150). The #6150 DO sensor is fitted with a #6155 membrane that must be kept moist at all times. If the membrane is allowed to dry out, it can be rehydrated by immersion in a water bath at 50 ± 5 ℃ for 24 hours. Take precautions to be sure the water bath does not expose the sensor membrane through evaporation during the rehydration. Aging DO sensors and membranes can show a tendency to read "0.00" mg/L for a minute or more after turning on before reading correctly. When this symptom occurs, the next steps are to rehydrate or replace the membrane, followed by sensor replacement.

<u>Temperature Check</u>

The 6560 temperature sensor is reported to not require calibration and there is no mechanism available to calibrate or adjust temperature performance. Project experience has found the sensor to be reliable for many years of service. Despite this, confirming temperature sensor performance is essential because all other sensor measurements are temperature compensated. Pre- and post-deployment checks should be made with a NIST traceable thermometer (accurate to ±0.2 ºC). Fill a bucket of water in the laboratory and allow the bucket to sit for ≥1 hour. Record temperature with the YSI sensor and certified thermometer at the same bucket depth. The YSI sensor is acceptable if the measurement is within ± 0.3 ºC of the certified thermometer. If a certified thermometer is not available, use a bucket of crushed ice in distilled water to check temperature accuracy. Allow the ice and water to acclimate for 20 minutes and immerse sonde. The sensor is acceptable if the measurement is 0 ºC ±0.3.

Temperature Troubleshooting. Circuit disruption or contamination (typically grit or water) on the temperature port connector can cause poor temperature sensor performance. This has been observed when dirt on the connector causes an unusually high temperature reading. This error can be confirmed by removing the probe from the sonde and checking the temperature display. Any reading other than -9.99 °C indicates that connector contamination and/or an interruption between the batteries and sensors has occurred.

<u>Pressure/Depth Calibration</u>

The sonde should be set horizontally on laboratory bench (not immersed in water) and calibrated to 0.00 M. Alternatively, the pressure sensor is the only sensor that can be calibrated in the field by holding the sonde at the water surface and calibrating to 0.00 m. No additional calibration procedures are needed for the depth sensor module used in freshwater applications. See the YSI manual for barometric pressure and salinity considerations when seeking accurate depth measurements for marine applications.

<u>pH Calibration</u>

Calibration. Conduct a two-point calibration using pH buffer standards that are certified traceable to NIST with an accuracy of ± 0.05 pH. Always use 7.00 pH for the first standard during calibration and select 4.00 or 10.00 depending on the expected pH range of water at your station. Allow at least one minute of temperature equilibration for each buffer. Record pre- and post-calibration values and pH mV for both buffers. The YSI recommended mV range for YSI 6561 pH probes in 7.00 buffer is ±50.

pH Troubleshooting. It is not uncommon to see the YSI 6565 pH sensor produce mV readings > ±50 mV in 7.00 pH while maintaining calibration within specified data quality objectives. New probes tend to track near 0 mV and range higher with age. With each calibration, record mV at 7.00 pH and watch for unstable readings. When mV measurements first exceed ±50 at 7.00 pH, or when fluctuations are first noticed, calculate the sensor's slope by recording the pH mV for both buffers. The span between the 7.00 pH mV and second buffer reading is the sensor slope and the acceptable range is 165-180. Sensors that are out-of-range for pH 7.00 mV or slope can be reconditioned by soaking the sensor overnight in 2 M KCL solution or 1 hour in 1M HCL followed by 1 hour in tap water. Sensors that are stable, maintain calibration and are within diagnostic ranges can continue to be used. Sensors that continue to have out-of-range mV and/or unstable readings should be replaced.

Specific Conductivity

Calibration. The conductivity sensor is reported by YSI to be linear for the specified range of 0-100 mS/cm. Therefore, only a single point calibration is needed with a standard in the range of the sampling station's specific conductivity. Standard solutions should be traceable to NIST standards and have a stated accuracy of ≤1% of the standard concentration. An acceptable alternative to commercial standards is to prepare your own starting with a stock 1.0 M KCL solution. If preparing a KCL standard, the user should follow instructions from MassDEP's SOP on Water Quality Multi-probes (MassDEP 2005) and must have access to high quality DDW. Acceptable standards for freshwater sampling range from 1.0 mS/cm for freshwater to 50.0 mS/cm for marine waters. The high linearity allows flexibility; however, the selected standard should be near the range of sampled waters.

Conductivity Troubleshooting. The YSI conductivity probe has proven to be reliable and consistently holds calibration for long deployments. To minimize temperature compensation error, calibrations should be conducted at stable room temperature near 25 ºC. Be aware of incorrect readings during calibration caused by air bubbles trapped in the conductivity cell or from having too little standard solution to cover the entire cell. Add more solution or gently move the sensor up and down to remove the bubbles. If calibration or field measurement errors are suspected, the conductivity cell constant can be checked in the Advanced Menu under "Cal Constants". The acceptable range is 5 ± 0.45. Values outside this range point towards a problem with the sensor or calibration solution. The sensor can be further checked by removing it from the sonde and reviewing the conductivity reading. Values of 0.00 ± 3 uS/cm are acceptable and values outside this range indicate that sensor or port connectors are contaminated and must be cleaned. Soapy warm water is used to clean the sensor and connectors. For severe contamination, soak the sensor in hot, soapy tap water for one hour; followed by DDW rinses and air drying.

Salinity

Salinity readings are derived from the YSI's measurements of conductivity and temperature. No calibration is required for salinity measurements. However, the user should recognize that the algorithm for deriving salinity is linear for all measurements of conductivity. Therefore, salinity concentrations will be assigned for low levels of conductivity even when sampling is conducted in freshwater with no saltwater present. This feature may require the attention of users when urban deployments are near the salt and freshwater interface. The response in these cases could be to require data corrections for false low salinity readings or to ignore the parameter in freshwater.

Turbidity

Calibration Standard. The two-point calibration recommended by YSI for the YSI 6136 sensor uses DDW as the 0.0 NTU standard and 126.0 NTU polymer-based standard manufactured by YSI. Other commercial turbidity standards are available, but not recommended. The DDW should come from a high-quality laboratory system documented in Section 2.0 Technical Notes. Turbidity standards should be stored away from direct light in a constant temperature setting. *Option:* although 126.0 NTU is preferred and recommended as the second standard, because of the high cost, 126.0 NTU standard can be reduce volumetrically to a 10.0 - 30.0 NTU standard using DDW water.

Calibration. First, run a wiper cleaning cycle to be sure the wiper does not park on the optic port. Next, the two-point calibration should be conducted using the black bottomed, extended calibration cup with the cup resting on the laboratory bench and the sonde clamped to a laboratory stand with the probes pointing downward into the cup. The sonde bulkhead should rest on the top thread of the calibration cup. Start the calibration with the 0.0 NTU standard and be sure to rinse twice with DDW water. Be aware of bubbles or the wiper blocking the turbidity sensor and causing erroneously high values. Next, conduct the second point of calibration with 126.0 NTU.

Attention should be paid to sources of calibration error for 0.0 NTU standard. The sensor can detect low levels of contamination that are introduced to the 0.0 NTU standard from the calibration cup and sensors. The calibration of the slightly contaminated standard can create a positive offset of about 0.2 to 0.8 NTU. Conversely, if a standard-sized calibration cup is used instead of an extended cub, the sensor will be too close to the bottom of the cup and result in a slight negative bias in the calibration. This can reduce field measurements by approximately 0.5 NTU. If these conditions occur, YSI recommends (Mike Lizzote, YSI, *pers. comm*. Nov. 2008) the following method for correcting the offset at 0.0 NTU for 6920 V2s following the two-point calibration:

Place the sonde with sensor guard attached into a 3-5 g bucket of DDW that has settled for one hour. Once the turbidity sensor is acclimated, record the turbidity value, and conduct a one-point calibration for 0.0 NTU. This will account for both the calibration cup and contamination error.

Turbidity Troubleshooting. Turbidity sensor failure is usually first indicated by poor or failed post-calibration performance. There are few diagnostic checks available to the user to evaluate the turbidity sensor. You can cover the turbidity sensor with your finger and should see a reading of 1000-1400 NTU. If the sensor does not respond to your finger, the probe has failed and must be returned to YSI for reconditioning or discarded. Be aware during review of continuous sonde data that very high NTU values can be caused by debris blocking the sensor. *Option:* if available, a laboratory bench-top turbidity meter can be used to check YSI probe performance with standard solutions.

When using 6136 turbidity sensors with 6920 sondes, be sure that the sonde and 650 display firmware are upgrades to Version 3.10. For unattended sampling, the turbidity time constant should be increased from the factory setting of 12 seconds to 30 seconds (menu: Advanced/Data Filter/Time Constant). This will improve the sensor's stability at low turbidity measurements. For all uses of 6136 sensors, be sure to use black-colored turbidity wiper mounts or blacken white wipers with paint or markers. Users of 6600 sondes should consult with the YSI manual to account for the different calibration cup size from the 6920 sonde.

Deployment Procedures

Initiate Unattended Logging. Once calibration is complete, follow YSI manual instructions to initiate unattended logging. Logging sampling frequency is dependent on project, and typically 15, 30, or 60 minutes. Specific projects should select a consistent sampling frequency. The logging interval is dependent on battery capacity, sampling frequency and sensor performance. Deployments of 3-4 weeks are suitable for most projects. Intervals that exceed 5-6 weeks run the risk of losing battery power or DO membrane (6562) failure. Assign a file name for each deployment that contains the site name and a three-digit year/deployment code (ex. Jones19-1). Verify correct date and local standard time, parameter setup, and start logging. Be sure to activate pH mV and DO charge in Report Set-up. In Advanced Set-up, activate Auto sleep RS232 and SDI-12 functions with a 60 second interval for DO warm-up.

Sonde Preparation and Deployment. The sonde anchor should be streamline and allow the sonde to sit parallel to flow without high visibility. The anchor should allow the sensors to sit at least two inches above the substrate to avoid interactions with sediment and algae. A 30-50 lb. cut-off section of railroad track is a good platform to use as an anchor. The sonde can be attached to the anchor with black tie-wraps and duct tape to reduce visibility. Wrap the sonde with a cloth rag before applying duct tape. Insert a business card or agency ID in waterproof sleeve into the exterior wraps of duct tape. The sonde and anchor should have low visibility once sitting on the stream bed. Orient the sonde so the sensors face downstream to avoid catching debris on the sensor guard.

Post-Deployment Procedures. Following every retrieval of sondes, QA/QC checks and post-deployment calibration must be conducted in the laboratory with the sonde acclimated to room temperature. If the sonde will be redeployed and has a 6562 DO sensor, then planning must allow for a DO membrane change. In this case, the best approach to avoid losing chemistry readings for a calendar day is to retrieve the sonde in the afternoon and conduct the precision checks, DO and turbidity post-deployment checks, and membrane change that afternoon. The remaining post-deployment calibration can be conducted the next morning before redeployment.

Download Data. Download data to YSI 650 display or PC. Briefly view field data to confirm a successful deployment and to flag compromised data or sensors that have potentially failed.

DO Post-Deployment Check. The DO 6562 sensor performance should be checked before cleaning or changing the membrane. Repeat the pre-deployment test for DO. This reading will serve as the post-deployment check for DO. Following the test, the DO membrane should be changed. The following morning the DO probe should be calibrated again to set the calibration for the new membrane. For sondes with the optical DO probe, only a single calibration is needed and can be done in sequence with the other parameters.

Turbidity Post-Calibration Check. Inspect the sensor face to identify and record evidence of biological fouling. Remove wiper and thoroughly clean all sensors. Reinstall the wiper and verify that it is parking correctly. Prior to the two-point calibration, conduct an "after cleaning" check with DDW. Record this value (should be near 0.0 NTU) for as an indicator of sensor performance.

Post-Deployment Calibration. This step is crucial because it will provide the information needed to evaluate the quality of the logged data and serve as the pre-deployment calibration for the next deployment. Proceed with the calibration using the same protocols as during pre-deployment. Conduct additional sensor and sonde bulkhead cleaning if there is evidence that the sensors are not residue-free following the initial cleaning and two DDW rinses.

Battery Changes. In most cases, batteries will be replaced with each deployment. Batteries can be redeployed if voltage is >11.5 V for deployments in warmer weather. Decisions on changing batteries should consider temperature, sampling frequency and deployment duration.

QUALITY ASSURANCE AND CONTROL

There are two processes for reviewing and validating YSI multi-probe water chemistry data. The first process is to export YSI EcoWatch data to Excel and review the timing of raw data to flag potential outliers. Use the records in Form 2.1 on calibration and deployment timing to trim "out-of-water" data. Secondly, the pre- and post-deployment calibration data are reviewed to identify if the data are within acceptable ranges of accuracy and precision. Once these protocols are completed, the data can be adjusted if needed and classified.

Data Documentation. Raw water chemistry data are saved in an annual Excel datafile that is named after the river sampled and year (e.g., Jones River-2020). The following three worksheets in this file contain water chemistry data: *raw data*, *final data*, and *daily mean*. The Excel datafile also contains three additional worksheets used for calibration and QA/QC review. The first form, Calibration Form 2.1 will be a printed form used in the laboratory during calibrations. The user can keep a paper file for Form 2.1 or elect to enter data into the worksheet file. Data from Form 2.1 is next transcribed to Form 2.2 which is used to review all calibrations for that season. The third form is Form 2.3, which summarizes all calibration and QA/QC procedures for the sampling season and classifies the data. Forms 2.2 and 2.3 will be maintained only as electronic files.

Database Management. Data files will be saved on the common server (W:\) and back-up files will be saved on the primary server (P:\) of the Database Manager. The data classification will be updated by the QA/QC Analyst and care should be made to ensure the back-ups are consistent with the primary files. Once all possible review is completed and data has received the final classification, the annual river datafile will be saved as read-only files in both the common and primary servers.

<u>Datafile Review</u>

Deployment Schedule. The *raw data* worksheet in the annual Excel datafile should be reviewed to confirm that deployment time, retrieval time, and the sondes internal clock are consistent with Form 2.1 records. Make notes in the *raw data* worksheet to indicate the start and end of each deployment. Copy *raw data* to the worksheet named *final data* and trim data that were recorded before or after the sonde was placed in the river.

Outlier Review. Scan the data for each deployment in the *raw data* worksheet for outliers and evidence of failed sensors. Make notes on obvious problems. The most common errors found to date are failing DO sensors (usually sensor *#6562* membrane damage) and debris blocking the turbidity sensor. Highlight potential outliers and return to these questionable data once you have summarized the calibration. With the exception of salt wedge influence on conductivity, and debris blocking turbidity optics, most outliers are caused by probe failure and will be flagged during post-deployment calibration.

Turbidity Outlier Troubleshooting. Some data outliers are easily flagged as errors and others are measurements that could occur naturally without clear indication that these marginal values should be removed. Debris covering the turbidity sensor can produce a high turbidity reading near the sensor maximum (1000-1400 NTU). If a single reading in that high range occurs with base flow turbidity on either side of the measurement, this outlier should be censored from the *final data* worksheet. The difficulty comes with random spikes over 100 NTU during rain events. Some of these higher readings could be a partially blocked sensor. It is recommended that turbidity data from all annual deployments in a river are reviewed at the same time to develop an understanding of base flow conditions. A calculation should be made of each river's mean turbidity during base flow (no precipitation on day of sample and two previous days) for each season. Base flow values that are 3x the nearest value and ≥3 SD of the mean base flow should be classified as *Conditional* and scrutinized as potential outliers. The decision to *Censor* turbidity outliers will be made by the QA/QC Analyst after considering the distribution of available station data on turbidity.

DO Outlier Troubleshooting. In the case of the 6562 DO sensor, a breached membrane will cause a slow, but apparent reduction of the DO charge (and DO concentration). The post-deployment calibration will confirm the membrane has failed. The QA/QC Analyst must then review the data stream to decide at what time the membrane failed and *Censor* the subsequent data.

<u>Calibration Review</u>. For each deployment, the pre-deployment and post-deployment calibration results will be evaluated for data classifications. The QA/QC Analyst should complete the Calibration Review Form 2.2 and assign a preliminary status for each parameter on the basis of the calibration results. Calibration results for each parameter in Form 2.2 will be classified as *Accept*, *Conditional*, or *Censor*. Following a review of the *raw data* worksheet, the QA/QC Analyst summarizes calibration results and other deployment checks on Form 2.3 to classify all river data for the season.

If sensor data has acceptable calibration accuracy deviations, precision checks, and outliers, then the data can be accepted as *Final*. Data that exceed the allowable deviations will be classified as *Conditional* (shade worksheet cells yellow) data and subject to further review. Next, field data collected between calibrations should be reviewed for outliers and deviations in expected baseline conditions for that river. If no aberrations are found the field data will be classified as *Conditional*. A warning limit of ≥ 3 SD for the station time series for each parameter will trigger a decision by the QA/QC Analyst. Field data that exceeds the warning limit and are associated with calibration or precision errors will likely be C*ensored.* Form 2.3 should document causal factors for deviations and outliers and provide concluding comments on the decision to censor the data or keep it as *Conditional*.

Censored data should be shaded red in the *final data* worksheet and not transferred to the *daily mean* worksheet. With the exception of the turbidity sensor, *Censored* data will most often be associated with a failed sensor. Overall, the most common occurrence of accuracy deviations and outliers among sensors will be for turbidity.

Datafile Classification. The QA/QC Analyst should review the data and classify the QA/QC review status and data status using the classes listed below. The QA/QC status refers to the review stage for the entire datafile. The data status refers to the status of data under the QA/QC review. This datafile classification is consistent for all SOPs under this QAPP.

QA/QC Status

1. Draft. Data processing is in progress, and QA/QC has not been conducted.

2. Preliminary. Data processing is complete, but QA/QC is not complete. Data can be used for internal project summaries.

3. Complete. All data processing and QA/QC review is completed.

Data Status

1. Preliminary. Data have been entered from field sheets or downloaded but QA/QC review is not complete.

2. Censored. Data are eliminated because of instrument failure or QA/QC performance.

3. Conditional. Data are fully audited, and QA is complete, but have deficiencies that are documented and may limit use.

4. Final. Data are fully audited, checked and Acceptable for all uses.

Linear Adjustment of Data. The YSI operation manual does not offer suggestions for adjusting data following the identification of QA/QC problems. In some cases, you will not be able to identify a causal factor for a sensor failing calibration or for outliers. When a successful pre-deployment calibration is followed with post-deployment concerns there may be evidence of an error in post-calibration procedures or steady directional drift in measurements. For example, during post-deployment calibration the turbidity sensor could measure 2.0 NTU lower than the 0.0 NTU standard and the raw data consistently has base flow values lower than expected and some negative values. In this situation, linear adjustment may be appropriate if a calibration error can be identified. The condition should be recorded in Form 2.3 and these data would remain *Conditional* but could be used for daily mean data. Linear adjustment will only be permissible when a clearly identified calibration error influenced a sensor's performance in a linear manner for an entire deployment.

Field Precision Measurements. There is no requirement to assess the precision of replicate measurements in the field for long-term deployments. Parameter precision will be measured with each pre-deployment and post-deployment calibration in the laboratory. This decision was made because of the high precision observed to date with program laboratory measurements and the inconvenience of recording 2-minute precision checks on sondes programmed for deployment and secured to anchors. *Option:* if questions develop over the precision of field measurements, the sonde can be activated for deployment after recording replicate measures at 2-minute intervals on station. Another option is to record replicate measurements for two or more program sondes placed side-by-side at the sampling station before and/or after deployments. The acceptable RPD and RSD for both these measures of precision is ≤ 5% (and 25% for turbidity).

SINGLE POINT MEASUREMENTS

The YSI sondes are also used to collect individual, single point measurements or grab samples at river, lake and marine sampling stations. The collection of grab samples requires the user to follow all sampling and calibration protocols applicable from the YSI operations manual. The user should also follow all calibration, deployment and storage procedures from the Long-Term Deployment section of this SOP with the following exceptions or additional steps for single point measurements.

Calibration

Calibration Frequency. When possible, calibrate the sonde on the day of sampling. This is not always practical or necessary for some applications. At a minimum, calibration should be conducted on the first day of sampling during a given work week and continue during the sampling season on a weekly basis. We calibrated YSI sondes on a daily basis for many years and found this high calibration frequency was not necessary to maintain sensor performance specifications nor cost effective. *Option:* if post-deployment calibrations identify concerns with weekly calibrations application, the calibration frequency can be increased. Most likely, this concern would involve the DO sensor. A simple solution is to calibrate DO independently each day of sampling. An on-site check can be made for the DO #6562 sensor by wrapping the sensor guard in a water-soaked towel and running the sonde for 10 minutes to confirm that the DO value is within specifications. If the reading is out of tolerance, then simply recalibrate on-site. This check is not needed for the optical sensor and only an option for the #6562 sensor because vibrations and temperature swings can affect the Teflon membrane and cause sensor drift. In practice, we have found little concern over post-calibration drift with careful sonde treatment and weekly calibrations.

DO Sensor. Make sure the AutoSleep RS-232 function has been turned off for grab sampling. This function is found by following the sonde's Setup Menu to Advanced Menu.

Temperature Check. The accuracy of the temperature sensor should be checked weekly during the calibration bucket precision check with a NIST traceable thermometer.

Sample Collection

Sample Location. A sample location should be designated and recorded in GPS at each river or pond station, and consistently used. River locations should have an identifiable landmark and receive mixed flow from the stream channel. Most sampling under this SOP will be in the spring. Minor changes in sampling location caused by sampling during summer low flows are acceptable when documented.

Water Column. The water column depth where measurements are recorded should be standardized for each monitoring project. Surface measurements for rivers and lakes should be recorded at a depth that exposes the sonde cable connector to air and places the sensors at a depth of approximately 0.3 m. In shallow streams (<0.3 m) the sonde can be rested horizontally on the bottom when hard substrate is present or tilted at an angle with the sensor guard on the bottom and the upper sonde resting on a stable surface (rock, branch, sampler's boots) that is downstream of the sonde and does not interfere with stream flow.

Acclimation Time. The acclimation time for sensors to settle on accurate values is primarily dependent on temperature. To be consistent, for the first sample at a given station, allow at least 10 minutes of acclimation time for all grab measurements when water temperature ≥5 °C. Changes in water pH and DO between stations can also influence response time for those probes, especially in cold water. The acclimation time should be increased to 15 minutes for water temperature <5 °C. A 10-minute acclimation time may appear too conservative for summer sampling; however, stratification in lakes can slow sensor response when passing through the thermocline. This SOP update allows a reduction of the 10-minute acclimation rule to 5-mintues during summer sampling when the sonde can rest in pond water (bucket or attached to boat gunnel) between stations. Additional water column measurements can be taken after a 5-minute acclimation period, providing that the sonde did not pass through the thermocline to reach the next sample. In all cases, monitor the display to determine when the sensors have stabilized. Sondes that rested in a warm car for a long drive may need a few extra minutes to acclimate in cold water.

Multiple Samples. For QA/QC purposes, replicate measurements will be made to assess sampling precision. All river station measurements should be made in triplicate at two-minute intervals. For lakes, where multiple water column measurements are made, a duplicate measurement is sufficient to check field sampling precision. A surface sample duplicate should be made at a 2-minute interval for one station per lake per sampling trip.

Quality Assurance. The review of sonde calibrations and precision checks will follow the same process as for unattended logging. Deviations from accuracy specifications and parameter RPDs ≥5% will result in classifying sensor data as *Conditional*. The warning limit for turbidity RPD is set higher at 25% because of the common occurrence of deviations from equilibrium for low turbidity concentrations in a flowing stream.

Data Recording. Field data should be recorded to the YSI 650 display to a file designated for the given river and year. The file can be downloaded at the end of the season to Ecowatch. It is an option for each participant and user to also transcribe the data to a field sampling sheet (not supplied in QAPP) as a safeguard against data loss. With experience and careful attention, we have found that paper records as back-ups have not been necessary. Secondly, the use of replicate sampling to assess precision creates an onerous transcription effort.

Data Documentation. Raw data should be transcribed or downloaded to an annual Excel datafile that contains data for all river stations. Grab sample calibrations are documented on Form 2.4 and QA/QC is evaluated by transcribing weekly calibration data to Form 2.2. Form 2.3 will not be necessary for grab sample data.

Database Management. Use the same procedures as with Long-Term Deployments.

STORAGE AND TRANSPORTATION

During the sampling season, instruments should be transported and stored in a carrying case. The case should be cushioned to prevent movement of the sonde during transport. The calibration cup should cover sensors with a third volume of tap water. After each use, the sonde (with calibration cup on) and display unit should be allowed to dry on the bench top. After each marine deployment, all components should be cleaned with tap water. The carrying case should be cleaned and set out to dry on a weekly basis.

MAINTENANCE

With-in Season. It should not typically be necessary to remove sensors from sonde during with-in season maintenance for freshwater deployments. A test-tube brush or toothbrush is suitable for dislodging sediment and organic deposits. The probes can be soaked briefly in warm, soapy water or white vinegar prior to cleaning. With each cleaning between long-term deployments inspect conductivity ports, DO anodes, and the glass bubble of pH sensor and refer to YSI operational manual for specific cleaning instructions. The turbidity wiper pad should be removed and cleaned (or replaced) following each long-term deployment.

Sonde, Sensor, and Cable Connectors. Be careful not to drop the disconnected cable connectors into dirt or hard surfaces. With careful use, the cables will perform well for many years. The problems associated with small amounts of contamination on the connectors are easily avoided. Erratic readings can be associated with dirt or water on the connections. If this occurs, the connectors can be cleaned with warm soapy water applied from a squirt bottle. Following cleaning, dry the connectors thoroughly with air pressure or a hair dryer.

Annual Maintenance. At the end of the sampling season, remove all sensors and clean o-rings. Sensors should be cleaned and stored dry, except for pH and DO sensors. The 6562 DO sensor should be fitted with a new membrane and stored in tap water, and the DO optical sensor is also stored in water. The pH sensor should be refurbished every year of use (max. of 2-3 years) by storage in 2 M KCl, followed by an hour soak in bleach to kill bacteria and an overnight soak in tap water. The pH sensors can be stored for a month or less in tap water, but never in distilled water and should not be allowed to dry out. Replace any o-ring that shows the slightest sign of wear. When sensors are re-installed for the start of the sampling season, lubricate all o-rings with a light application of silicon grease.

TECHNICAL NOTES

YSI Calibration Tips. Mike Lizotte of YSI produced a document in 2009 with tips on calibrating YSI 6-Series sondes (Lizotte 2009). This document is a valuable supplement to YSI manuals. Users of this QAPP can obtain a copy from the authors. Further, YSI has several valuable Technical Notes on 6-Series sondes and sensors available on their website (https://www.ysi.com/customer-support/resource-library).

Distilled-Deionized Water. Distilled water can be used for standard preparations if the laboratory distiller provides high grade distilled deionized water. The distiller and deionizer should be listed in the SOP with specifications for resistivity and annual maintenance. If high grade DDW can be achieved and maintained, then the DDW can be used to prepare pH buffers, 0.0 NTU standard, and to prepare turbidity standard dilutions. If the laboratory precision check uses DDW the specific conductivity should be recorded and compared to specifications.

Program Distillers. The DMF program participants will use DDW from two sources at the Anniquam River Marine Fisheries Station in Gloucester and the SMAST West Campus in New Bedford. Both water systems were custom fabricated for our laboratories by from Industrial Water Technology and produce a DI water resistivity of >18 Megohms. The water systems were purchased for Gloucester in 2012 and for New Bedford in 2007. Both systems are professionally service 2-3 times annually.

Specific Conductivity. The conductivity sensor accuracy specifications (2002 and 2005 manuals) are ± 0.5% plus 0.001 mS/cm of reading when properly calibrated. Our experience has found this probe to be one of the most consistent and durable YSI sensors; however, a quality control problem exists since slight deviations from low concentration standard solutions will exceed the accuracy level. Post-deployment calibration violations are more likely for freshwater applications than marine. These specifications have changed over time, as evident from the 1997 YSI 6820 manual that reported a sensor accuracy of ±5% from standard conductivity solutions. For this project, we will adopt a ±2% deviation from standard solutions as acceptable accuracy. Deviations from ±2-5% will result in a *Conditional* classification for data, and data that exceed ±5% will be *Censored* unless a calibration error provides justification for linear adjustment.

The remaining three categories in Technical Notes are not SOP recommendations. They are references on evolving alternative methods that can be applied by project partners in a troubleshooting mode or considered for future QAPP versions.

Dissolved Oxygen. The YSI protocols for calibrating DO probe#6562 have been tuned over the years with increasing experience. Recent suggestions allow for pre- and post-deployment calibration of the DO probe while the sonde is in logging mode with a sample frequency of 15 minutes or less. This option is an acceptable alternative to the protocol under DO Calibration (6562 sensor). To calibrate while logging, allow the sonde to record data for 2 hours before deployment with the calibration cup set for DO calibration. After 2 hours, calibrate the DO probe. With this process, you will have water-saturated air data recorded. The post-deployment check is done in the same manner while the sonde is still logging. This approach provides a longer record of data to evaluate DO sensor drift which may benefit some applications.

Air-Saturated Water. YSI recommends using air-saturated water as an alternative method for calibrating DO sensors. Allow a 5 g bucket of water to aerate for at least 1 hour with an aquarium air pump and air stone. Place the sonde with sensor guard attached into the bucket to acclimate for 10 minutes and calibrate to 100% saturation. The air-saturated water calibration requires more time than the water-saturated air calibration but is reported to create less opportunity for error. Both methods can produce the same results if done correctly. This method is not recommended as the primary approach for DO calibration for this SOP due to of concerns over consistency among program participants that could result in calibration saturation values that are under or over 100%.

Low Ionic Standards. MassDEP recommends a protocol for using quality control standards for pH and conductivity when sampling low ionic waters (MassDEP 2005). A low-ionic phosphate standard stock solution can be prepared to confirm sensor performance.

LITERATURE CITED

Lizotte, M. 2009. Calibration tips for YSI 6-Series Sondes & Sensors. January 2009, Yellow Springs Incorporated, Yellow Springs, Ohio.

MassDEP. 2005. Standard Operating Procedures for Water Quality Multi-probes. CN:004.21. Mass. Dept. of Environ. Protection, Div. of of Watershed Mgt., Worcester, MA.

YSI. 2006. 6-Series Multiparameter Water Quality Sondes User Manual. Revision D, October 2006, Yellow Springs Incorporated, Yellow Springs, Ohio.

Form 2.1. YSI 6-Series Sonde Long-Term Calibration Form.

YSI 6-Series Sonde -- Form 2.1 for Long-Term Deployments

STEP 1: PRE-CLEANING CHECK

LOCATION:
SONDE ID:
DATE: TIME: INITIALS:

Parameter	Pre-Cal Reading	Post-Cal Reading
DO Sat%		
DO Charge (6562 only)		
Turbidity (0.0 NTU)		

Time (in): ____________
Time (out): ____________
Clock Check: ____________
(correct/DST)

STEP 2: PRE (or POST)-DEPLOYMENT CALIBRATION

DATE: TIME:

Parameter	Pre-Cal Reading	Standard Used	Post-Cal Reading	RPD (1)	RPD (2)	RPD (%)
Temperature (ºC)						
Sp. Cond. (mS/cm)						
DO Sat% (new mem.)						
pH (1)						
pH (2)						
Turb. (1) (NTU)						
Turb. (2) (NTU)						
Depth (m)						
pH mV (for pH7)						
DO Charge (new mem.)						
mmHg						

Battery Charge (V) pre/post change: ____________
Wiper Service: ____________
New Filename: ____________

NOTES:

STEP 1: PRE-CLEANING CHECK

LOCATION:
SONDE ID:
DATE: TIME: INITIALS:

Parameter	Pre-Cal Reading	Post-Cal Reading
DO Sat%		
DO Charge (6562 only)		
Turbidity (0.0 NTU)		

Time (in): ____________
Time (out): ____________
Clock Check: ____________
(correct?/DST)

STEP 2: POST-DEPLOYMENT CALIBRATION

DATE: TIME:

Parameter	Pre-Cal Reading	Standard Used	Post-Cal Reading	RPD (1)	RPD (2)	RPD (%)
Temperature (ºC)						
Sp. Cond. (mS/cm)						
DO Sat% (new mem.)						
pH (1)						
pH (2)						
Turb. (1) (NTU)						
Turb. (2) (NTU)						
Depth (m)						
pH mV (for pH7)						
DO Charge (new mem.)						
mmHg						

Battery Charge (V) pre/post change: ____________
Wiper Service: ____________
New Filename: ____________

NOTES:

Form 2.2. YSI Sonde Calibration Summary Form.

MASSACHUSETTS DIVISION OF MARINE FISHERIES

Form 2.2
YSI Sonde Calibration Review

Location:
Year:

File Type:
Sonde ID:
QA/QC Analyst:
QA/QC Status:
Data Status:

Pre-Deployment

Date:

(1)

Parameter	Units	SOP Specs. (±)	Pre-Calibration	Standard	Post-Calibration	Deviation from Spec.	Precision Lab. (%)	Notes
Temp.	*(ºC)*	0.3						
DO	*(% sat.)*	5%						
Sp. Cond.	*(mS/cm)*	2%						
pH (1)		0.2						
pH (2)		0.2						
Turbidity (1)	*(NTU)*	2.0 or 5%						
Turbidity (2)	*(NTU)*	2.0 or 5%						
7.0 pH mV		(-50 to +50)						
DO charge		<75						

Summary:

Post-Deployment Calibrations

Date:

(1)

Parameter	Units	SOP Specs. (±)	Pre-Calibration	Standard	Post-Calibration	Deviation from Spec.	Precision Lab. (%)	Notes
Temp.	*(ºC)*	0.3						
DO (pre)	*(% sat.)*	5%						
DO (post)	*(% sat.)*	5%						
Sp. Cond.	*(mS/cm)*	2%						
pH (1)		0.2						
pH (2)		0.2						
Turbidity (1)	*(NTU)*	2.0 or 5%						
Turbidity (2)	*(NTU)*	2.0 or 5%						
7.0 pH mV		(-50 to +50)						
DO charge		<75						

Summary:

Notes:

1.) Add extra numbered and dated tables for each weekly or post-deployment calibration.
2.) Remove "DO (post)" and "DO charge" rows for calibrations with optical DO probe (no probe membrane).
3.) Classify each parameter as *Accept, Conditional* (highlight cell yellow) or *Censor* (highlight cell red).
in deviation from specifications and laboratory precision columns.

Form 2.3. YSI Sonde Calibration QA/QC Form.

MASSACHUSETTS DIVISION OF MARINE FISHERIES

Form 2.3
YSI Sonde QA/QC Review

Location:
Year:

Deployment Notes:

File Type:
Sonde ID:
QA/QC Analyst:
QA/QC Status:
Data Status:

PRE-DEPLOYMENT CALIBRATION

	Calibration Accuracy Spec. Dev.	**Precision Lab. RPD** (%)	**Data Status**	**Notes**
WaterTemp.				
DO				
Sp. Cond.				
pH (1)				
pH (2)				
Turb. (1)				
Turb. (2)				
7.0 pH mv				
4.0 pH mV				
DO charge				

Summary:

POST-DEPLOYMENT: Time and Battery Check

	Pre-Deployment	**Deployment No. 1**	**Deployment No. 2**
Deployment Time:			
Retrieval Time:			
Internal Clock Time:			
Battery (pre/post V):			
Notes:			

QA Review	**Deployment No. 1**				**Deployment No. 2**			
	Calibration Accuracy Spec. Dev.	**Precision Lab. RPD** (%)	**Precision Field RPD** (%)	**Data Status**	**Calibration Accuracy** Spec. Dev.	**Precision Lab. RPD** (%)	**Precision Field RPD** (%)	**Data Status**
Temp. *(ºC)*								
DO *(% sat.)*								
Sp. Cond. *(mS/cm)*								
pH (1)								
pH (2)								
7.0 pH mV								
4.0 pH mV								
Turb. (1) *(NTU)*								
Turb. (2) *(NTU)*								
DO charge								

Outlier Review:		
Data Adjustments:		
Summary:		

Form 2.4. YSI Sonde Single Point Calibration Form.

MASSACHUSETTS DIVISION OF MARINE FISHERIES Form 2.4

YSI 6-Series Sonde - Calibrations for Single Point Sampling

DATE: **TIME:** **INITIALS:**

PROJECT: **QA STATUS:**

Variable	**Pre-Cal Reading**	**Standard Used**	**Post-Cal Reading**	Relative Percent Difference **(1)**	**(2)**	**RPD (%)**
Temperature (ºC)			NA			
Sp. Cond. (mS/cm)						
DO Sat.%						
pH (1)						
pH (2)						
Turb. (1) (NTU)						
Turb. (2) (NTU)						
Depth (m)						
pH mV (7.00 / 4.00)						
DO Charge						
mmHg:						

Sonde ID:
Battery (V):
DDW Sp. Cond:
DO Membrane Change? (Y/N):

Notes:

DATE: **TIME:** **INITIALS:**

PROJECT: **QA STATUS:**

Variable	**Pre-Cal Reading**	**Standard Used**	**Post-Cal Reading**	Relative Percent Difference **(1)**	**(2)**	**RPD (%)**
Temperature (ºC)			NA			
Sp. Cond. (mS/cm)						
DO Sat.%						
pH (1)						
pH (2)						
Turb. (1) (NTU)						
Turb. (2) (NTU)						
Depth (m)						
pH mV (7.00 / 4.00)						
DO Charge						
mmHg:						

Sonde ID:
Battery (V):
DDW Sp. Cond:
DO Membrane Change? (Y/N):

Notes:

SCOPE AND APPLICATION

This appendix to Section 2.0 of the QAPP's Standard Operating Procedures (SOP) provides guidance for operating the YSI 556 Multi-Point System (MPS) water chemistry instrument. The monitoring and data quality objectives for this application are described earlier in SOP 2.0. The purpose of this SOP is to mainly document the instrument's specifications and provide guidance on calibrations and maintenance. This document does not cover all aspects of instrument calibration and operation. It is important that users of the YSI 556 MPS become familiar with the YSI operations manual (YSI Multi-Probe System Operations Manual, 2014). These protocols offer additional points of clarification on YSI manual instructions and quality control and assurance procedures specific to our applications monitoring diadromous fish habitat.

Monitoring Objectives. The YSI 556 MPS will be deployed to record single-point water chemistry measurements within coastal river watersheds monitored for diadromous fish resources. The recorded data will assist ongoing fisheries sampling programs and interagency efforts to manage aquatic resources. The protocols can also be applied to DMF projects measuring water chemistry related to other aquatic marine resources and habitats.

INSTRUMENTS

Yellow Springs Incorporated developed the YSI 556 MPS nearly 20 years ago as a lower cost, light-weight alternative to the 6-series sondes for water chemistry measurements. The YSI 556 MPS measure temperature, pH, DO, conductivity and derive salinity from conductivity. MA DMF deploys a single YSI 556 MPS purchased in 2014 (SE# 14A100143) and maintained at our South Coast Marine Fisheries Station in New Bedford.

SPECIFICATIONS

Sensor resolution, range, and accuracy are provided by the manufacturer for each measured parameter (Table 3A.1). These specifications represent a baseline of expected performance and criteria for evaluating calibration results. It is our experience that properly calibrated and functioning MPSs will provide results within these specifications, with few exceptions.

Data Quality Objectives. The same data quality objectives listed in Table 1.1 will apply to YSI 556 applications. Water quality data within the accuracy range specified by YSI for each parameter probe should be attainable with accurate and consistent calibration. The SOP adopts all accuracy specifications directly from the YSI Multi-Probe System Operations Manual. These accuracy objectives can be monitored by conducting and reviewing pre-deployment and post-deployment calibrations. Precision checks of sensor parameter measurements are conducted in the laboratory during each calibration by recording the relative percent difference (RPD = (difference of two consecutive measurements / average of two consecutive measurements) x100).

Precision Check. Allow a bucket of tap water to acclimate to room temperature (≥1 hour). Place the MPS sensors in the bucket and allow sensors to acclimate to water temperature for at least 10 minutes. At this point, record water chemistry on Form 2.4 and repeat measurements after two minutes. At this time, also check the temperature sensor against a National Institute of Standards and Technology (NIST) traceable thermometer.

CALIBRATION

Calibration Frequency. When possible, calibrate the MPS on the day of sampling. This is not always practical or necessary for some applications. At a minimum, calibration should be conducted on the first day of sampling during a given work week and continue during the sampling season on a weekly basis. *Option:* if post-deployment calibrations identify reduced accuracy with DO sensor, the calibration frequency for DO should be increased to daily.

Calibration Containers. The transport cup used to protect the sensors serves as a calibration cup for all calibrations and reduces the volume of calibration standards used. Alternatively, laboratory glassware can be used for calibrations. Perform calibrations with the probe sensor guard installed.

Pre-calibration rinses. Prior to running calibrations for each sensor, the sensors should be rinsed with the calibration standard to be used. This is done for pH and conductivity. In the case of DO, rinse twice with DDW water. After each calibration, rinse the sensors with DDW water before proceeding with the calibration standard rinse. To reduce costs, the standard rinses should use standard solution saved from previous calibrations. The rinse solution should be discarded after one use.

DO Calibration

DO Membrane Cap. The YSI 556 MPS DO sensor uses the YSI 5909 DO membrane kit that contains membrane caps for the sensor. There are three options for membrane caps related to DO response time and flow dependency. The three cap options are color coded and have specific codes that must be designated in the Sensor menu of the YSI 556 MPS software when calibrating. Users should be familiar with these options by referring directly the Operations Manual.

Dissolved oxygen can be calibrated using one of two options (% or mg/L). Calibrating using either option automatically calibrates for both units. It is recommended to calibrate using % saturation. Prior to calibrating, the instrument must be on for at least 20 minutes to polarize the DO sensor. When ready to calibrate (Menu, Calibrate), select "Dissolved Oxygen" in the Calibrate Screen. Conduct DO calibration in % saturation mode. Place approximately 3mm (1/8 inch) of water in the bottom of the calibration cup, then place the sensors into the calibration cup making sure that the DO and temperature sensors are not immersed in the water. Screw the calibration cup to the sonde only 1-2 threads as air space is needed to vent with the atmosphere. Enter the local barometric pressure as indicated on the DO Sat Calibration Screen. Allow 10 minutes for the air in the calibration cup to become water saturated and for the temperature to equilibrate. The values of all enabled sensors will stabilize during this period. When the DO% value remains stable for 30 seconds, press ENTER. The MPS screen will indicate that the calibration has been accepted.

DO Troubleshooting. Problems with the DO sensor are likely detected by unstable or inaccurate readings. Significant errors will produce an *out-of-range* message on the display during calibration. These symptoms can be the result of a variety of factors. The sensor should be properly calibrated under the correct temperature and Barometric pressure in accordance to the DO calibration procedures in the YSI 556 MPS Manual. The DO membrane should be inspected for damage and to make sure it is properly installed. If damaged, the membrane cap should be replaced. In addition, the DO sensor should be inspected to ensure there is no water in the sensor connector, algae or other contaminants. If tarnished, the DO silver anode and gold cathode can be cleaned following specific instructions in YSI 556 MPS Operations Manual. After cleaning, a new membrane cap should be installed.

Erratic or unlikely DO measurement values can often result from a degraded membrane cap or deficient KCl electrolyte solution. The KCl solution should be changed at least every 30 days and at any indication of poor DO measurement performance. If changing the KCl electrolyte does not improve performance, then the membrane cap should be replaced. Dissolved oxygen measurements with the YSI 556 MPS consume DO during operation. This requires stirring the water sample at the sensor tip at a rate of at least 1 ft/s. If the user forgets to stir the sample or stirs at too slow a rate the DO concentration can be erroneously low.

Specific Conductivity Calibration

A single point calibration for specific conductivity calibrates both specific conductance and salinity for the model 556 MPS. In the Calibrate Menu, select "Conductivity," then "Specific Conductance" to display the conductivity calibration screen. Place the specified volume of conductivity standard the calibration cup (Table 3A.2). The conductivity standard should be within the same conductivity range as the samples being measured. However, standards less than 1.0 mS/cm are not recommended. Make certain the oxygen and pH/ORP sensors are free of salt deposits. Insert the sensors into the calibration cup with solution (with the sensor completely immersed past the vent hole), screw and tighten. Using the keypad, enter the calibration value (in mS/cm at 25°C) of the conductivity standard used. Press ENTER to display the Conductivity Calibration Screen and allow at least one minute for temperature equilibration and values of enabled sensors to stabilize. When the Specific Conductance value is stable for 30 seconds, press ENTER. The screen will indicate that the calibration has been accepted. Once the calibration has been accepted, return to the Calibrate Menu.

Conductivity Troubleshooting. Problems with the conductivity sensor are likely detected by unstable or inaccurate readings. Significant errors will produce an *out-of-range* message on the display during calibration. Care should be taken to ensure the sensor is cleaned and calibrated according to protocols in the YSI 556 MPS Manual. Calibration solutions should be within the required specifications and free of contaminants. The sensor should be inspected for damage and to ensure it is clean. If damaged, the sensor should be replaced.

<u>pH Calibration</u>

As with YSI Series 6 sondes, pH should be calibrated by conducting a two-point calibration using pH buffer standards. The first calibration should be conducted using a pH 7.00 buffer, then use either a 4.00 or 10.00 buffer depending on the expected pH range of water during monitoring. In the Calibrate Menu, select “pH” to display the pH calibration screen. Under the pH Calibration Menu, select the 2-point option. Place the specified amount of buffer into a clean, dry or pre-rinsed calibration cup (Table 3A.2) and completely immerse the sensor into the solution. Securely tighten the calibration cup to the sonde. Use the keypad to enter the value of the first pH buffer standard (7.00) at the current temperature (pH vs. temperature values are printed on the labels of all YSI pH buffers). Press ENTER to access the pH Calibration screen and allow at least one minute for temperature equilibration and values for all enabled sensors to stabilize. When the pH reading is stable for 30 seconds, press ENTER, and the screen will indicate that the calibration has been accepted. Press ENTER and repeat the same procedure for the second pH buffer.

pH Troubleshooting. Problems with the pH sensor are likely detected by unstable or inaccurate readings. Significant errors will produce an *out-of-range* message on the display during calibration. Care should be taken to ensure the sensor is cleaned and calibrated according to protocols in the YSI 556 MPS Manual. The sensor should be inspected for damage and to ensure it is clean with no water in the sensor connector. If damaged, the sensor should be replaced.

If the pH sensor has been allowed to dry out for short periods of time it can be restored by soaking in 2 M KCL or standard solution 4.0 for several hours or overnight. If this treatment does not result in an accurate pH calibration the sensor needs to be replaced.

MAINTENANCE

Sensor Storage. The temperature and conductivity sensor should be brush cleaned before storage; and can be stored wet or dry. Tap water is suitable for wet storage. The pH sensor must be stored in water. Use tap water and do not store the pH sensor in DDW.

LITERATURE CITED

YSI. 2002. YSI 556 Multi Probe System Operations Manual. May 2002. Yellow Springs, Inc., Yellow Springs, Ohio.

Table 3A.1. YSI 556 MPS Specifications (YSI 556 MPS Operations Manual;YSI, 2002). Refer to Table 1.1 for standard data quality objectives for YSI 556 applications.

Dissolved Oxygen	
Sensor Type	Steady State Polarographic
Range: % air saturation (SAT) mg/L	0 – 500% SAT 0 – 50 mg/L
Accuracy: % SAT mg/L	0 – 200% SAT (±2% of reading or 2% SAT; whichever is greater) 200 – 500% SAT (±6% of reading) 0 – 20 mg/L (±2% of reading or 0.2 mg/L; whichever is greater) 20 – 50 mg/L (±6% of reading)
Resolution: % SAT mg/L	0.1% SAT 0.01 mg/L
Temperature	
Sensor Type	YSI Precision™ Thermistor
Range:	-5 to 45°C
Accuracy:	±0.15°C
Resolution:	0.01°C
Conductivity	
Sensor Type	4-electrode cell with auto-ranging
Range:	0 – 200 mS/cm
Accuracy:	±0.5% of reading or ±0.001 mS/cm (whichever is greater – 4m cable) ±1.0% of reading or ±0.001 mS/cm (whichever is greater – 20m cable)
Resolution:	0.001 mS/cm to 0.1 mS/cm (range-dependent)
pH	
Sensor Type	Glass combination electrode
Range:	0 – 14 units
Accuracy:	±0.2 units
Resolution:	0.01 units
Barometric Pressure	
Range:	500 – 800 mm Hg
Accuracy:	±3 mm Hg within ±15°C temperature range from calibration point
Resolution:	0.1 mm Hg

Table 3A.2. Calibration volumes for YSI 556 MPS (YSI 556 MPS Operations Manual; YSI 2002).

Sensor to Calibrate	Upright	Upside Down
Dissolved oxygen (DO)	N/A	3 mm (1/8 inch)
Specific Conductivity	55 ml	55 ml
pH/ORP	30 ml	60 ml

Section 3.0 Rainbow Smelt Spawning Habitat Assessment

SCOPE AND APPLICATION

Rainbow smelt (*Osmerus mordax*) are an anadromous fish native to the Atlantic coast of North America. Smelt are an important forage fish for many species of wildlife and supported traditional commercial and recreational fisheries in New England that have declined in recent decades. The declining fisheries trend and reduced presence in Southern New England prompted the National Marine Fisheries Service (NMFS) to designate smelt a "Species of Concern" in 2004 under their review process for the Endangered Species Act.

Section 3.0 of QAPP V-1 was developed to guide DMF's work delineating smelt spawning habitat and to serve the efforts of the states of Maine, Massachusetts, and New Hampshire that worked cooperatively during 2008-2012 under a grant from the NMFS Protected Species Division to develop a conservation plan to prevent further reductions in New England smelt populations. This updated Section 3.0 under QAPP V-2 contains minimal revisions. The 2008-2012 smelt conservation plan project has been completed (Enterline et al. 2012). DMF continues to measure water chemistry at smelt spawning habitat stations with no changes to SOP protocols. It was decided to include SOP 3.0 in QAPP V-2, despite the few changes and conclusion of a major objective, in order to have all QAPP SOPs in one document.

Smelt spawning in New England occurs during the spring freshet in March-June. Spawning habitat is typically found at gravel and cobble riffles upstream of the tidal interface. Smelt deposit demersal, adhesive eggs that incubate in spawning riffles for 1-3 weeks, depending on water temperature. The reproductive strategy of having an extended incubation for stationary eggs may be susceptible to reduced success if the spawning habitat is degraded. Land use and hydrologic alterations can increase stream vulnerability to impacts from nutrient enrichment, reduced shading and riparian buffer, and non-point source pollutants. Watershed alterations in Massachusetts have contributed to spawning habitat degradation from physical alterations, reduced flow, sedimentation, eutrophication, and acidification (Chase 2006). Eutrophication may be the primary source of degradation in urban watersheds by causing excessive periphyton growth in spawning riffles. These concerns have also been raised for smelt runs in tributaries to the St. Lawrence River in Quebec (Lapierre et al. 1999). Field observations in Massachusetts indicate that high periphyton growth at spawning riffles causes reduced smelt egg survival (Chase 2006). However, relationships between water quality, smelt spawning habitat degradation, and smelt populations have not been assessed. More information is needed on the condition of smelt spawning habitat in New England and influences on habitat quality.

The influence of nutrient pollution on water and habitat quality in rivers and lakes is a growing concern in the United States (US EPA 1998; Mitchell et al. 2003). The trophic state of a river is influenced most by light, carbon sources, nutrients, hydrology and food web structure (Dodds 2007). Among these influences in developed watersheds, nutrient enrichment is most dependent on human activity and may be most amenable to remediation efforts. The US EPA recommends that States develop nutrient water quality criteria that can be used to protect specific designated uses of aquatic habitat under Clean Water Act (CWA) assessment and remediation processes (US EPA 2000a). This approach depends on setting criteria or reference conditions for causal and response variables that can act as thresholds for protecting designated uses. The reference conditions will represent minimally impaired water quality and are based on the lower 25th percentile of a statistical distribution of causal and response variables. Section 3.0 adopts the US EPA recommended approach for developing water quality criteria for smelt spawning habitat with the goal of producing an assessment tool that can contribute to Clean Water Act processes and protect smelt spawning habitat throughout the species range. Smelt spawning habitat will be assessed with three approaches in Section 3.0: spawning habitat delineation, field measurements of water quality and primary productivity, and the application of water quality criteria.

<u>Monitoring Objectives</u>. The main purpose of Section 3.0 is to provide standardized protocols for delineating and assessing smelt spawning

habitat and to develop habitat assessment tools within the framework of US EPA and *Mass*DEP CWA guidelines. The following objectives should improve our understanding of the negative and positive influences on water and habitat quality at smelt spawning habitat and provide valuable information for the resource management goals of protecting and restoring anadromous fish habitat and enhancing smelt populations.

1. Delineate and document river and stream locations where smelt spawning occurs.

2. Select fixed sampling stations at smelt spawning habitat where biotic and abiotic parameters related to spawning habitat will be measured. Identify water and habitat quality deficiencies at each station using physical, chemical and biotic criteria.

3. Develop reference condition thresholds and relationships between abiotic conditions and measures of primary productivity.

4. Incorporate monitoring results into CWA processes for protecting designated habitat uses and make recommendations for improving and protecting specific locations of spawning habitat.

<u>Reference Conditions</u>

Nutrients. The US EPA's Nutrient Criteria Technical Guidance Manual for rivers and streams (US EPA 2000a) recommends several statistical approaches for developing nutrient criteria for total phosphorus (TP), total nitrogen (TN) and chlorophyll *a* (chl *a*). In the absence of data on reference conditions for protecting designated uses, US EPA recommends using the 25th percentile of the distribution of measured variables from a population of rivers within a region. The 25th percentile serves as a threshold between degraded locations and minimally impacted reference locations. The US EPA has generated reference conditions using the median of the four seasonal 25th percentiles for all rivers sampled in the Northeastern Coastal Zone (Ecoregion 14, sub-region 59; US EPA 2000b). Nutrient data collected under this SOP will be compared to these thresholds during the assessment of the trophic status of each sampling station. In addition, independent reference conditions will be calculated from the 25th percentile of data collected during the smelt spawning season for TN and TP. These data will contribute to habitat assessments and the development of designated use criteria for smelt spawning habitat.

Physico-Chemical. The US EPA recommendations for nutrient criteria do not include criteria for water chemistry response variables such as dissolved oxygen and pH. Thresholds for designations of suitable spawning habitat will be adopted from *Mass*DEP's Surface Water Quality Standards (SWQS) for temperature, DO, and pH. These thresholds along with physical thresholds for spawning habitat were investigated under the smelt conservation plan project. All reference criteria are presented in Table 3.1.

Algal Biomass. Periphyton (also referred to as benthic algae) biomass is a useful indicator of water quality because it is sessile, fast growing and relies on the water column for uptake of nutrients and minerals. The US EPA nutrient recommendations include reference conditions for phytoplankton chl *a* but not for algal biomass in the stream bed. Although there is less guidance for algal biomass, the percentile distribution approach used for nutrients can also be applied to algal biomass. Riskin et al. (2003) used a median concentration for periphyton biomass of 21 mg/m^2 as a mesotrophic (moderately enriched) threshold for New England streams. The value was derived from a summary of published studies on nutrient and periphyton relations (Biggs 1996). The 50th percentile of algal biomass data collected under this SOP can be evaluated as the mesotrophic threshold. Furthermore, the 25th percentile can be evaluated as a threshold for reference streams and the 75th percentile can be evaluated as a threshold for impaired streams.

<u>Hypothesis</u>. Smelt spawning habitat monitoring in Massachusetts resulted in a hypothesis that states a primary threat to smelt populations is the degradation of spawning habitat from watershed pollution (nutrient, sediments, contaminants) and alterations (flood control and transportation structures, land development, and dams) (Chase 2006). Specific to eutrophication, it is hypothesized that elevated nutrient concentrations have degraded spawning habitats by enhancing

periphyton growth and reducing the suitability of spawning substrate for egg survival.

Watershed Classification. Sampling stations are in coastal watershed basins of the Gulf of Maine in the subecoregion 59 of the Northeastern Coastal Zone (US EPA 2000a). All stations should be initially classified by the following three watershed categories (US EPA (2000a).

1. Non-assigned streams (not assessed by State waterbody assessments).

2. Impacted streams (on States 303(d) list or designated as impaired in 305(b) reports).

3. Reference stream that are minimally impacted; with the following three conditions: a.) watersheds with <5% impervious surface cover; b.) watersheds with <5% agricultural use and <5% of disturbed riparian buffer; c.) watersheds with population densities <20 people per square mile.

Artificial Substrates. Artificial substrates have been used extensively in water quality monitoring to relate periphyton growth and species composition to ambient water quality, although concerns remain over the reliability of measurements (Weitzel 1979; and Lowe and Pan 1996). When using artificial substrate to collect periphyton for this application, three assumptions are made: (1) all substrates deployed have equal colonization and development of periphyton, (2) sample replicates are exposed to identical conditions, (3) changing water chemistry is the only variable influencing periphyton growth and species composition at the different sampling locations. If these conditions can be met, substrata can be sampled for indirect measures of periphyton productivity (ash-free dry weight (AFDW), biovolume, and chlorophyll) and species community. Clearly, natural variations in the conditions of water velocity, depth, shading, grazing, scouring and solar incidence can challenge these assumptions. The careful development of sampling design, site selection, and application of QA/QC procedures are essential to successfully relate periphyton sampling to water and habitat quality.

Data Quality Objectives. Parameter-specific data quality objectives are presented in Table 1.1. For water chemistry parameters measured with YSI sondes, these objectives are provided and discussed in SOP Section 2.0 and adopted for all projects under the QAPP. The primary data quality objectives are based on accuracy and precision. Accuracy objectives are derived from the manufacturer's sensor specifications and are monitored by conducting and reviewing calibrations. The precision of sensor measurements is monitored in the field and laboratory by recording the relative percent difference (RPD) of two consecutive readings or relative standard deviation (RSD) of three or more consecutive readings.

Nutrient and periphyton biomass data objectives are specific to SOP Section 3.0. Additional details, including laboratory specifications for each parameter are provided in the QA/QC section of this SOP. In addition to specific warning limits for accuracy and precision, data quality objectives are provided for reviewing outliers. Several biotic and physical parameters listed in Table 3.1 are not included in Table 1.1 because the existing information on smelt spawning habitat is too limited to define numeric criteria. The application of Section 3.0 may provide data that will improve numeric criteria for smelt spawning habitat.

MATERIALS

Artificial Substrate. Unglazed ceramic tiles will be used as artificial substrate for periphyton collection. We have had success using the "Mayflower Red" flat quarry tile and recommend this type. The tiles are purchased as 6x6 inch squares (15x15 cm or 0.0225 m^2). When cut into four squares, the tile area is 7.4x7.4 cm (0.00548 m^2). A copper wire ring will be attached with marine epoxy to the bottom of each tile to hold a hooked metal rod as a substrate anchor.

Drying Ovens. A drying laboratory oven capable of maintaining a constant temperature of
105 ºC is needed for periphyton samples and a muffle furnace capable of constant temperatures ≥500 ºC is needed to ash periphyton samples.

Analytical Balance. A high-quality balance is needed to weigh AFDW samples. The balance should be readable to 0.0001 g and calibrated annually to maintain an accuracy of ±0.0005 g.

Aluminum Weight Boats. Aluminum weigh boats should be used for holding the periphyton samples during the drying process and should be specified to tolerate temperatures >500 ºC.

Desiccator. A glass desiccator capable of holding up to 35 aluminum weight boats will be needed for holding periphyton samples prior to weighing.

Water Chemistry Equipment. A multi-probe water chemistry sonde is needed for continuous logging or grab samples at the tile stations. The sonde should meet SOP specifications.

Water Velocity Meter. A stream flow velocity meter is needed for weekly flow and depth measurements at the tile station. The meter should operate over a velocity range of 0.1 to 3.0 m/s and have a resolution of 0.01 m/s. A meter stick is needed to measure water depth (cm).

Scraping Tool. A flexible, synthetic scraping tool should be used to remove periphyton from tiles. These tools are commonly sold for marine fiberglass application. The scraper width should cover the tile width (at least 7.4 cm). The scrapers should be purchased in bulk for all project and replaced when the blade becomes worn.

Smelt Egg Scoop. A stainless-steel autoclave basket (approximately 12x12 cm) attached with hose clamps to a solid wood broom pole. This egg scoop is well-suited for checking gravel in riffles for the presence of smelt eggs.

Global Positional System (GPS). A hand-held, battery-operated GPS unit is needed for recording smelt spawning habitat and sampling station locations.

DELINEATION OF SPAWNING HABITAT

The level of effort needed for delineating smelt spawning habitat and selecting spawning habitat sampling stations will depend on existing knowledge in each region. Observations of deposited eggs formed the basis for documenting smelt spawning habitat. Smelt migrate during evening high tides to freshwater riffle habitat where they deposit demersal, adhesive eggs. In relatively large smelt runs, deposited smelt eggs are readily found at the first freshwater riffle upstream of the tidal interface. In rivers where smelt spawning habitat has been documented, additional effort on mapping spawning habitat may not be necessary and the program participant can proceed to site selection in the following section on Tile Deployment.

In rivers where information is lacking on the spatial extent of smelt spawning habitat, it is recommended that the following methods from Chase (2006) are used to confirm the presence of smelt spawning and to document spatial and temporal spawning habitat use. In the target watershed, all freshwater drainages should be surveyed for potential smelt spawning habitat. Locations that contain suitable freshwater riffles can be selected for routine monitoring. Smelt spawning habitat is considered the river water column and substrate where smelt egg deposition is observed. Potential smelt spawning habitat is defined as habitat that possessed suitable riffles to attract smelt spawning but either was not previously known to be smelt spawning habitat or no egg deposition was observed during study monitoring. The physical and chemical conditions that provide suitable spawning habitat are not well documented. Table 3.1 contains a list of parameters that are important for the attraction of spawning adults and smelt egg survival.

Each selected monitoring station should be visited at least twice a week for the entire duration of the smelt spawning period to inspect stream substrata for the presence of smelt eggs. Cobble should be inspected by hand to look for smelt eggs and a smelt egg scoop can be used to inspect gravel. Egg monitoring should initially focus on the first riffle found upstream of tidal influence.

The identification of the first riffle typical requires several reconnaissance visits to the location at low and high tide stages. Once egg deposition is identified, monitoring should expand to nearby riffles until the upstream and downstream limits of egg deposition is recorded. A monitoring log should be maintained with each station visit to record qualitative observations and GPS locations on the spatial extent of spawning locations. Eggs are identified on the basis of size, oil globule, and seasonal comparison with other species (Cooper 1978; Elliot and Jimenez 1981). Depending on the smelt run size and spatial extent of spawning habitat, the delineation may require 1-3 seasons. One season of monitoring should be sufficient to allow the selection of a riffle station for habitat assessments under this SOP. Customized monitoring strategies will be needed for rivers that

Table 3.1 Physical, Chemical, and Biotic Criteria for Smelt Spawning Habitat. The water chemistry parameters relate to Massachusetts SWQS for protecting aquatic life at Class B Inland Waters (*Mass*DEP 2007), and US EPA reference conditions for the Northeast Coastal Zone sub-Ecoregion (US EPA 2000b).

Variables	**Suitable** (SWQC or BPJ)	**Minimally Impacted** (25th percentile)	**Notes/Source**
CHEMICAL			
Temperature (ºC)	≤ 28.3		Maximum limit (*Mass*DEP 2007)
Temperature (ºC)	≤ 20.0		7-day mean of daily max. (*Mass*DEP 2007)
pH	≥ 6.5 to ≤ 8.3		(*Mass*DEP 2007)
DO (mg/L)	≥ 6.0		(*Mass*DEP 2007)
Turbidity (NTU)		≤ 1.7	EPA Ecoregion 14, sub-59 (US EPA 2000b)
TN (mg/L)		≤ 0.57	EPA Ecoregion 14, sub-59 (US EPA 2000b)
TP (ug/L)		≤ 23.75	EPA Ecoregion 14, sub-59 (US EPA 2000b)
PHYSICAL			
Substrate Size (Ave. mm)	>2.0		Chase (2006)
Water Velocity (Ave. m/s)	0.3 - 1.0		Chase (2006)
Slope (%)	0.5 - 1.0		Chase (Pers. obsv.)
Riffle	Presence/Absence		Best Professional Judgment (BPJ)
Canopy			BPJ based on percent open canopy
BIOTIC			
Aquatic Moss	Presence/Absence		BPJ
Periphyton Biomass ($g/m^2/d$)			Not defined
Phytoplankton Chlorophyll (ug/L)		≤ 0.44	EPA Ecoregion 14, sub-59 (US EPA 2000b)

are not safely wadeable. Very few smelt spawning runs in Massachusetts are not wadeable. The few exceptions were monitored with additional methods such as setting ichthyoplankton nets and deploying egg collection platforms attached to anchors and buoys (Chase 2006).

TILE DEPLOYMENT

Update Note: Tile deployment for periphyton sampling was a component of QAPP V-1 that guided Smelt Conservation Plan efforts during 2008-2012. These protocols are repeated here in QAPP V-2 for continuity but are not being applied presently by an ongoing DMF project.

Site Selection. Site selection will be critical for meaningful comparisons and will take careful consideration because of the natural variation found in riverine habitats and the common presence of tidal influence. All sampling stations should be active smelt spawning riffles that were previously identified or delineated. Channel width should be close to 20 m (± 10 m) to allow wadeable access and have similar conditions of depth (0.5 m, ± 0.3 m), water velocity (0.5 m/s, ± 0.3 m/s) and canopy (no vertical cover bank-to-bank March-April). It is recommended that all stations be located in the freshwater zone in close proximity (<0.5 km) to the freshwater/saltwater interface. However, some rivers have dams near the tidal interface that cause greater fluctuations in depth and velocity and prevent smelt from passing further upstream. To account for this, each station should be ranked as either freshwater zone (no tidal influence during spring), tidal interface (moderate changes in depth/velocity with no salt wedge) or tidal zone (substantial changes in depth/velocity and salt wedge presence). Samples collected in freshwater zones have the highest likelihood of producing periphyton growth that can be related to environmental and water quality conditions. Samples from the tidal zone will be exposed to greater changes in physical habitat, but in some cases may represent the only viable spawning habitat in the river system and water quality will still be dominated by freshwater discharge for most of the tidal cycle. The presence of USGS stream flow gage stations and previous or ongoing water chemistry sampling elevate the value of candidate stations.

Tile Sampling

Tile Placement. Once a spawning riffle has been selected based on physical criteria, the precise placement of tiles depends on finding a level surface that receives fully mixed river flow. This approach lends towards mid-channel locations and avoids the river edge. All tiles should sit level on the river bottom. It is appropriate to groom a patch of bottom with a rake to ensure the bottom is level. Tiles should be placed in two rows running parallel to flow. Adjacent tiles should not influence each other. This can be achieved with level placement and a space of 1-2 cm between tiles. The tiles should be inspected during weekly visit between deployment and retrieval, and disrupted tiles no longer suitable for sampling should be removed.

Tile Replicates. The number of tile replicates should exceed the total number of needed samples by approximately 50%. The higher number of tiles deployed than tiles needed allows the collection of a random sample and provides back-ups in case some tiles are disrupted.

*Duration and Frequency***.** The deployment of tiles should coincide with the spawning period of smelt. Weitzel (1979) recommends 2-week durations for tile deployments. We have had successful deployments for both two and three weeks. With three-week deployments, the threat of scouring and grazing increases. For smelt habitat applications, two weeks can be too brief when low growth persists in early spring. For present applications, the target duration will be three weeks with the option to pull the tiles after two weeks if an impending storm threatens to scour the tiles. Four deployments should be made during the period of March - June. The onset of tile deployments will depend on ice conditions and the smelt spawning period in each region.

Tile Retrieval. The tiles selected for AFDW, periphyton identification and benthic chlorophyll will be selected randomly before retrieval. Generate random numbers to match with numbers marked on the tiles and include several alternative numbers in case the selected tiles are disrupted. The tiles must be carefully removed from the substrate to avoid disruption of periphyton. In shallow or warm waters, retrieval by bare hand is the best approach. It may be necessary to use arm-length gloves or a modified tool such as barbecue tongs in deep or cold water. Retrieve all needed tiles and place them in a transport tray with a cover to avoid sunlight. Carefully transfer periphyton from the tiles to sample containers in the field immediately.

Periphyton Sample Processing

Ash Free Dry Weights. Five tiles will be sampled for AFDW in each river per sampling period. Tiles will be scraped in the field through a funnel into plastic storage containers with sealing lids. First, remove all periphyton from the tile edge facing the container. Next, make three uniform sweeps across the tile with the scraper, pushing periphyton from the tile surface into the container. Finally, use distilled water from a squirt bottle to rinse periphyton from scraper and funnel into the container and to clean materials between samples.

The containers will be placed on ice until processing later that day. Upon returning to the laboratory, the boats will be dried overnight at 105° C and weighed to a constant weight (weighed on three separate days with storage in desiccators). Once dry weights are measured, store samples in a freezer until ashing at a later date. Samples will be ashed for 1 hour at 500° C in a pre-heated muffle furnace and then re-wetted with distilled water and dried again at 105° C and weighed to a constant weight (APHA 1989). The analysis units will be AFDW g/m^2 and g/m^2/day. *Option:* samples can be frozen on the sample day for subsequent dry weight processing.

Periphyton Identification. One tile will be collected in each river per sample period for periphyton identification. These samples will be transferred from the tiles directly into 125 ml jars containing 90 ml of distilled water and 3 ml of "M^3" preservative (APHA 1989; see Technical Notes). Tile samples will be scraped into the jars with 10 ml of distilled water from a syringe to assist the transfer. Samples will be stored in the dark until processed for periphyton identification to the lowest possible genera. A single duplicate tile will be collected randomly per trip for QA/QC analysis.

Natural Substrate Sampling. In addition to tile sampling, periphyton will be collected from natural substrate to identify standing algal communities. Select five rocks that are representative of the riffle substrate within 10 m of the tile transect. Follow the methods ME DEP for Natural Substrate Sampling (Danielson 2006). The rock samples will be processed the same as tile samples with the exception of using a ½ inch metal scraper and a 1-inch diameter neoprene washer to outline the scraping surface.

Periphyton Chlorophyll a (Option). Samples of benthic chlorophyll growth on tiles provide a measurement of photosynthetic periphyton and allow the estimate of an autotrophic index when related to AFDW. One tile can be collected in each river per sample period for chlorophyll *a* analysis. The sample will be scraped directly into 50 ml centrifuge tubes with chilled 90% acetone and stored in the dark on ice until filtering later that day. Filtering should occur in near-dark conditions and the filter paper should be rolled into a small glass jar, covered with tin foil, and placed in the freezer. Chlorophyll samples should be run within three weeks of freezing.

Periphyton Identification

Microscope Analysis. Periphyton samples in 125 ml jars should be vigorously shaken and an aliquot should be drawn immediately with an eye dropper. Place a single drop of sample on a glass microscope slide and cover with slide cover. Using a research grade, light microscope, scan the viewing field with necessary magnification (10X, 20X, 40X and 100 X objective lenses) to become familiar with the taxa present. The sampler should develop an understanding of algae identification using the guides of Smith (1950), Prescott (1978) and Wehr and Sheath (2003). Begin counting diatoms and algae cells at the right middle margin of the slide cover and record by genera or taxa groups. Follow a parallel transect line across the slide until filling the targeted number of cells.

Cell Counting. Each algal filament or diatom cell is counted as one. This includes cells that are in the process of dividing and strings of colonial diatoms. This approach can be applied consistently and requires less judgment among samplers. Counting each observed cell or filament as one will under-represent filamentous algae, but the alternative of assessing biovolume is labor intensive. *Option:* more information can be gained on filamentous algae by recording average cells per filament from a sub-sample of observed filaments.

Target Cell Count Number. Rarefaction curves were plotted by DMF during pilot species identification efforts to determine an appropriate number of cell counts (Krebs 1989). The "short-count" method of Weitzal et al. (1979) was used to count 500 cells from a sample with tallies recorded at 50 cell increments. The rarefaction curves plotted from these data identified 350 as the count when 90% of all genera and groups that occur in a 500 count are present. Based on these results, we have selected 350 as the target number of cell counts.

Taxa Grouping. Most algae and many diatom specimens will be seen in sufficient detail to identify genera. In some cases, especially for diatoms in girdle view, it will not be possible to separate genera. The most common grouping will be "unidentified pennate diatom" (Chetelat et al. 1999). Secondly, colonial diatoms that can have rectangular shape in girdle view, such as *Eunotia, Fragillaria, Tabillaria and Synedra,* are often difficult to separate, particularly in degraded samples. These diatoms will be grouped as "unidentified colonial diatom". *Option:* if applicable, pennate-shaped diatoms can be further divided into two sub-groups of "Naviculoid-shaped diatom" and "girdle view diatom"

Counting with Image Analysis Pro Software. When using Image Analysis software, snap photograph frames (10X) along transect line. The selection of frames to snap should not be biased by visual observations. To avoid this bias, frames should be taken from the border of the previous frame or selected while moving the objective along the slide cover without viewing the PC monitor. The number of frames taken will depend on cell density. Count cells with the manual count feature and export data to an Excel file. Once reaching the target count of 350, finish counting and identifying all remaining cells in the last frame.

Counting through Microscope. If imagery software is not available, the microscope can be used to count and identify all cells through the viewing field. Proceed along the transect, taking care to line up viewing fields at the border of the previous field. Once reaching the target count of 350, finish counting and identifying all remaining cells in the last frame.

ENVIRONMENTAL DATA

Basic water chemistry, water flow, and nutrient data will be collected weekly during tile deployments. Because periphyton biomass growth will be evaluated in terms of weight/day, the data among deployments will be most comparable if the same day of the week is selected for sampling throughout the season. The sampling schedule for a three-week tile deployment would result in measurements on the day of tile deployment, the day of tile retrieval and twice during the two weeks between these events. Future applications can consider the need for separate field SOPs for the sampling of light intensity, water flow, and nutrients, and training for program participants.

Water Chemistry. Follow Section 2.0 procedures for calibration and QA/QC for grab samples with YSI 6-Series sondes. Measure the following parameters during each weekly visit to sample stations: water temperature (°C), DO (mg/l), DO saturation (%), specific conductivity (mS/cm), pH, and turbidity (NTU). Three measurements will be made immediately downstream of the rows of periphyton tiles (within 0.5 m) at a depth of 10-20 cm from the substrate. The 1st measurement should be made between the two-tile rows after a minimum of 10 minutes acclimation time. Dissolved oxygen and pH values should be monitored to be sure the sensors have stabilized after 10 minutes. The 2nd and 3rd measurements should be taken at two-minute intervals on both sides of a 0.5 m wide transect in which the 1st measurement marks the middle. The three measurements will be used for QA/QC evaluations and averaged for reporting.

Light Intensity. Nutrient concentrations and light availability can be the most important factors influencing primary production in shallow streams. Site selection protocols were designed to provide a standard canopy among stations. The approach supports the assumption that tiles at all stations receive similar solar incidence. However, variations in riparian tree canopy and water depth and color could cause differences in the amount of light reaching the tiles. Therefore, each station must record light intensity at the tile transect. Hobo Pendant light loggers are a suitable option for acquiring light data (lumens/m^2). Hobo Pendants should be activated at 15-minute intervals for monthly deployments and anchored to the substrate within 1 m upstream or downstream of the tile station. Periphyton will grow on the deployed Pendants and obscure the light measurements. The Pendants should be wiped clean of algae during each weekly visit. In addition, the percentage of open canopy can provide a second measure to compare light at each tile station. At each tile retrieval date, measure the left and right canopy angles with a handheld clinometer at the tile station to calculate the percentage of open canopy.

Water Velocity. A measurement of water velocity (m/s) over the tiles should be made with a professional grade current meter. Current meters are factory calibrated and cannot be readily recalibrated during field use. The current meter selected should have manufacturer's specifications for confirming acceptable operation and these steps should be stated in the following QA/QC section. Water velocity should be measured at the same sampling frequency as water chemistry and at the same location relative to tiles. Three water velocity measurements should be made 10-20 cm from the bottom along with total depth (cm) at the same tile transect used for water chemistry sampling. Do not use automatic readings for instantaneous measurements of flow; instead record average velocity over a 40 second interval.

Discharge (Option). Discharge measurements (m^3/s) are recommended when no USGS gauge station is present near the tile station. Discharge will be measured at a flow transect with uniform dimensions in close proximity to the sample station. The midsection method of the USGS (Buchanan and Somers 1969) should be followed. Under this method a minimum of 20 vertical measurements (40 seconds each) will be made along the cross-section at six-tenths of water depth. Conducting discharge measurements with each site visit will be time-consuming and may not be compatible with all river stations. An alternative method is to relate water stage height to discharge by developing a depth rating curve at a river station. The rating curve is made by taking 6-8 discharge measurements across a range of flows and recording a relative staff gage height. Once the rating curve is established, the staff gage height or depth can be recorded with each station visit and related to discharge.

Water Nutrient Measurements. Water samples for TN and TP will be collected at the same sampling frequency as flow and water chemistry. Nutrient samples will be collected at the tile deployment stations when no tidal influence is present. Collect samples in 60 ml HPDE collection bottles. The bottles should be dipped downstream of the tiles at the mid-transect point and draw water at 10-20 cm from the bottom. The bottles should be half-filled, shaken vigorously and rinsed three times before drawing a sample of 50 ml. The samples should be stored on ice in the dark until freezing later that day (<8 hours after collection).

All sample bottles and associated glassware used for nutrient sampling should be first washed with phosphorus-free detergent (ex. Liqui-Nox) and rinsed with tap water before sitting overnight in a 10% HCL bath. Upon removal from the acid bath, glassware should be rinsed five times with DI water. *Option*-- the collection of water column chlorophyll *a* would be a valuable addition to nutrient sampling. Chlorophyll *a* samples will require shorter holding times and specific handing procedures. If Chlorophyll *a* is collected, the sampling specifications must be outlined in an appendix to Section 3.0.

Nutrient Analytical Procedures

Total Nitrogen. Total nitrogen will be analyzed under contract with the Water Quality Analysis Laboratory of the Department of Natural Resources, University of New Hampshire, Durham, NH. Total nitrogen is measured by alkaline-persulfate digestion followed by colormetric analysis on a Smartchem autoanalyzer using methods from the USGS Water-Resources Investigations Report 03-4174 (USGS 2003). The WQ Analysis Laboratory does not have a holding time specification for TN because of its long-term stability when frozen. Projects should synchronize TN sample holding and laboratory delivery with TP samples. Nutrient QA/QC is reported in the following section.

Total Phosphorus. Total phosphorus will be analyzed under contract with the Lakes Lay Monitoring Laboratory of the University of New Hampshire, Durham NH. Total phosphorus is measured using the manual ascorbic acid method (Standard Method, 4500-P.E.; APHA 1989) with a Milton Roy Spectronic spectrophotometer. The maximum holding time (collection date to laboratory analysis while frozen) for TP at the Lakes Lay Monitoring Program is 90 days.

Expression of Data Concentrations. Water chemistry data should be expressed to the decimal place indicated by the parameter resolution under Specifications in Section 2.0. Velocity and flow measurements should be expressed as 0.001 m/s and 0.001 m^3/s, respectively, or to two decimal places when using US customary units. Nutrient measurements will be expressed to the significant figures specified by laboratory method detection limits. Nutrient analyte concentrations will be reported as mg/L or ug/L. Reporting datafiles will contain conversion tables for uM concentrations.

USGS Discharge Data. No instream discharge measurements are needed for tile stations with nearby USGS stream flow gage stations. Discharge data can be retrieved from USGS at their website (http://waterdata.usgs.gov). The daily discharge values used for analyses will be the average of all daily mean discharge measurements for each day the tiles were deployed. Water velocity must still be measured in the field because gage stations do not provide velocity data.

Weather Data. After the conclusion of the field season, average daily air temperature and total daily precipitation should be recorded from a nearby weather station reported by the NOAA's National Climatic Data Center (http://www.ncdc.noaa.gov/oa/ncdc.html).

Weather Classification. Sample collection dates will not be random or target designated weather. This is because weekly sampling depends on the tile deployment schedule and occurs during the specific spawning season of smelt. It is assumed that weekly measurements will capture typical weather and run-off conditions experienced during the spawning season. We will characterize all sample dates by the amount of recent precipitation using criteria for dry (<0.125 in), wet, (≥ 0.125 to 2.0 in) and flood (>2.0 in) weather for intervals of both 1-day (day of sampling) and 3-days (including the day of sampling). Record the presence of rain at the time of station visits to assist subsequent adjustments for cases when rain begins after the time of sampling.

QUALITY ASSURANCE AND CONTROL

Quality assurance and control protocols will be applied for each of the following data collections: basic water chemistry, water flow, water nutrients, and periphyton. The QA/QC review depends on three main components of performance criteria that target data quality indicators of accuracy and precision. The analysis of pre and post-deployment calibration data is used to evaluate accuracy, but only pertains to basic water chemistry measurements. The analysis of the similarity of replicates (laboratory, field and blanks), and outlier review will be conducted on each of the data collections outlined below.

Water Chemistry

Basic Water Chemistry. All instrument handling, calibration, and calibration data review procedures are outlined in Section 2.0. Once the calibration analysis has been conducted, the following criteria can be applied to classify field data. When the field season is complete, RSD will be calculated for all triplicate parameter measurements. All triplicates that have a RSD <5% will be accepted and the triplicate average will be used for the daily parameter measurement. A seasonal mean will be calculated for each river from all parameter measurements with RSD < 5%. A warning limit of ±3 SD from the seasonal mean will be used to flag potential outliers. All triplicates with RSD ≥5% will be classified as *Conditional* data and reviewed for outliers. Individual replicates should be *Censored* when they are identified graphically as outliers by exceeding the seasonal mean by 3 SD and when removed from their corresponding triplicates cause the remaining duplicates to have a RPD of <5%. The seasonal mean data for each river should be cumulative when multiple sampling seasons are available.

Turbidity Exception. The quality of turbidity measurements can be challenged by interference from suspended objects, natural variation in stream flow, and base flows with very low NTU values. Minor differences of low values can cause high RSDs. This is a function of proportional statistics and not necessarily related to precision. Therefore, turbidity quality control will follow different warning and control criteria. The warning criterion for turbidity is raised to 25% RSD. Triplicates with RSD ≥25% will be classified as *Conditional* data. Individual replicates will be *Censored* if they are identified as outliers by exceeding the seasonal mean by 3 SD and are also ±3x the closest replicate.

Temperature Exception. The same condition found for turbidity when minor differences at low values cause high RSDs also occurs for water temperature data when the temperature is close to zero. For water temperatures <1.0 °C, the RSD warning criterion of 5% will be relaxed and the replicates will be accepted if they do not vary by more than the sensor's SOP accuracy criterion (±0.3 °C). *Updated exception from QAPP V-1.*

Conductivity Exception. Conductivity values close to zero also exceed the 5% RSD with minor differences among replicates. When specific conductivity values are ≤0.100 mS/cm the RSD threshold is raised from 5% to 25%. *Updated exception from QAPP V-1.*

Stream Flow Data. Stream flow data collected from the three flow cells along a 0.5 m transect downstream of the tiles are not considered replicates. This is because true differences in water depth and velocity can be expected in turbulent riffles. The three measurements are taken to produce an average condition experienced by the tiles. Although the data are not replicates, the RSD should be calculated for flow and depth measurements and RSD ≥25% should trigger a review of the field data to see if a transcription error occurred or if one of the three measurements routinely had a strong negative or positive influence on the average values at a given river station. No data corrections are necessary following data review, although routinely high RSDs may be indicative of irregular substrate and/or an unsuitable tile station.

Flow Meter Check. Each flow meter used should have quality control checks specified by the manufacturer to confirm suitable performance. Flow meter calibration is not an option for most meters. All meters should be cleaned with warm water after each use and allowed to air dry before storing in carrying cases. DMF primarily uses Teledyne Gurley Price "bucket wheel" current meters. A weekly spin test should be conducted and recorded on Form 3.1 for Price meters; with the bucket wheel spinning freely for at least 1.75 min. If the meter fails a spin test, the meter should be disassembled, lubricated, and tested again. If it fails a second spin test, the pivot should be replaced, followed by another round of spin tests. Other types of current meters should be tested weekly according to manufacturer specifications.

<u>Nutrients</u> (Table 3.2)

Field Sampling. Each program participant will collect one field duplicate for TN and TP weekly or at a rate to meet a target of 10% of the total seasonal sample number. The selection of rivers for duplicate sampling will be made randomly. Duplicates will be used only for quality control purposes and not averaged for reported values. The first sample collected will be used as the parameter measurement unless rejected by QA/QC protocols. Monthly trip blanks (N = 3) comprised of laboratory DDW water treated the same as actual samples should be processed each season by each program participant.

Total Nitrogen Laboratory Analysis. The Water Quality Analysis Laboratory at UNH uses an EPA approved QAPP to guide all aspects of their water quality analyses (UNH 2008). The TN analysis follows the methods of USGS (2003). Quality control samples from standards are run every 10-15 samples with a minimum of two per batch (typically 40-55 samples). Instrument calibrations are performed at the beginning of each batch using standards made from reagent grade chemicals. Calibration curves are generally linear and made of 4-7 points. A laboratory reagent blank, laboratory fortified blank, and laboratory duplicate are run every 10-15 samples during each batch. The USGS (2003) TN analysis reference is available at:
http://nwql.usgs.gov/Public/pubs/WRIR03-4174/WRIR03-4174.pdf

Total Phosphorus Laboratory Analysis. The Lakes Lay Monitoring Laboratory of the University of New Hampshire uses an EPA approved QAPP to guide all aspects of their water quality analyses (UNH 2007). Quality control samples from standards are run every 10-15 samples with a minimum of two per batch (typically 40-55 samples). Instrument calibrations are performed at the beginning of each batch using standards made from reagent grade chemicals. Calibration curves are generally linear and made of 4-7 points. A laboratory reagent blank, laboratory fortified blank, and laboratory duplicate are run every 10-15 samples during each batch. The total phosphorus SOP is located in the appendix of the Newfound Lake Watershed Assessment at the following website:
http://des.nh.gov/organization/divisions/water/wmb/was/qapp/documents/newfound_appendices.pdf

Nutrient Quality Control Acceptance Limits. A seasonal mean for all rivers will be calculated from nutrient measurements with RSD <35%. Field nutrient duplicates with a RPD <35% and both measurements <2 SD from the season parameter mean (SPM) will be accepted. A higher warning limit of 50% will be used for low

Table 3.2. QA/QC and Analytical Specifications for Nutrient Parameters.

Laboratory Quality Control	**TOTAL NITROGEN**		**TOTAL PHOSPHORUS**	
Units	mg/L		ug/L	
MDL	0.01 mg/L		0.8 ug/L	
RDL	0.05 mg/L		2.0 ug/L	
	(Frequency)	**(Control Limit)**	**(Frequency)**	**(Control Limit)**
Field Duplicate	1/week	<35% RPD	1/week	<35% RPD
Lab. Duplicate	1/10-15	≤15% RPD	1/10-15	≤15% RPD
Quality Control Sample	1/10-15	≤15% from control	1/10-15	≤15% from control
Lab. Reagent Blank	1/10-15	MDL	1/10-15	MDL
Lab. Fortified Blank	1/10-15	MDL	1/10-15	MDL
Lab. Fortified Sample Matrix	1/batch	<85% or >115% recovery	1/batch	<85% or >115% recovery

nutrient concentrations (≤ 10MDL). Low concentrations duplicates with an RPD <50% and with both measurements <2 SD from the SPM will be accepted. Duplicates that exceed the warning limit with one replicate >2 SD from the SPM, the duplicate <2 SD from the SPM will be used for the parameter measurement. Field duplicates with an RPD of ≥35% will be evaluated for handling errors and graphically to identify outliers. If no problems are identified and both duplicates are <3 SD from the SPM, the duplicates will be accepted as *Conditional* data. All values ≥3 SD from the SPM will be identified as outliers. All information on outliers will be evaluated and documented for final classification. The SPM data for each river should be cumulative when multiple sampling seasons are available.

Periphyton Biomass

Tile Rejection. Some randomly selected tiles may be disrupted and should not be used to process samples. This can happen from river flow shifting tiles, debris or high flows scouring the tile surface, relatively high invertebrate grazing, and mishandling during retrieval. The sampler should anticipate these occurrences and look for these negative biases. All project samplers will receive field training for tile sampling that includes examples of disrupted tiles. With evidence of these biases, the tile should be rejected from sampling and substituted with a tile randomly selected prior to the trip as an alternative.

AFDW Adjustments. Organic materials other than periphyton can settle on the tile causing a positive bias to AFDW. Non-organic materials are not a concern since they are deducted from dry weights during AFDW processing. Adhesive smelt eggs and larval insects have been observed to settle on tiles and positively increase periphyton biomass estimates. Low numbers of smelt eggs and insect larvae should be removed with fine forceps from the sample before the first drying cycle. Large numbers of eggs or insects are more problematic and must be deducted from the sample weight if a suitable alternative tile is not available. Egg and insect weights can be measured by running subsamples of these organic materials through the AFDW process. Random samples of at least 10 eggs or larvae should be placed in four subsample weigh boats and included in a batch run of AFDW samples. From the subsamples, a mean weight per egg or larva should be calculated and used as a basis to deduct weight from AFDW samples that were run without removing large numbers of eggs or larvae.

Weigh Boat Blanks. Four aluminum weigh boats should be run as blanks with each batch of AFDW samples. The weigh boat weights would not be expected to change during drying or ashing. However, a negligible reduction in boat weight (0.0005 g) was recorded during one trial during QAPP V-1 pilot efforts.

Periphyton Acceptance Limits. Large variation among periphyton replicates is expected and may represent fine-scale differences in natural controls on periphyton growth (Weitzel et al. 1979; APHA 1989; Morin and Catteneo 1992; and Lowe and Pan 1996). A warning limit of 35% RSD is set for AFDW replicates. Replicates with ≥35% RSD should be scrutinized for disruption among individual samples during collection or drying. Replicates with ≥35% RSD with no evidence of disruption or outliers should be classified as *Conditional*. All samples that exceed the seasonal mean periphyton replicates by ≥3 SD will be classified as outliers. The outliers should be evaluated graphically and by reviewing the dry weight data, and field and laboratory data sheets. Marginal outliers with no evidence of handling disruption can be accepted as *Conditional* data and all others should be *Censored.*

Periphyton Identification. Quality control measures will be conducted to evaluate the precision of periphyton species identification among tile samples and within-sample jars. Data from each sample will be recorded by cell counts and relative percent abundance by genera or taxa group. The Bray-Curtis diversity index and Pinkham-Pearson coefficient of similarity (Weitzel et al. 1979) will be calculated for each sample to evaluate the similarity of samples. Each program participant will select weekly random tiles for duplicate periphyton identification or at a rate to meet a duplicate target of 10% of the total seasonal sample number. Periphyton identification will be done on single aliquots from the duplicate samples and a RPD of <35% for each index will be accepted. If either RPD is ≥35%, a third replicate should be identified. All triplicate samples with RSD of <35% will be accepted and all samples with an RSD ≥35% will be classified as *Conditional* data and reviewed by QA/QC Analyst for taxonomic errors. *Option:* precision among samplers can be assessed by drawing random triplicate samples from 10% of sample jars. Two samplers will identify periphyton from the same triplicate sample. Combined RSDs of <35% will be accepted for triplicate samples. Combined samples or individual sample RSDs ≥35% will be classified as *Conditional* data and trigger a review by the QA/QC Analyst for taxonomic errors.

Reference Conditions and Habitat Assessment

Percentile Distribution. Smelt spawning stations are sampled during the spawning period in March-May. A median value for water chemistry and nutrient parameters should be calculated for each river for each sampling season. The reference condition for the ME/NH/MA smelt Species of Concern project will be calculated by grouping all median values from rivers in the three states and calculating the 25th percentile from this distribution. The data should also be summarized annually by rivers with the following statistics: minimum, maximum, median, mean, standard error, and 25th, 50th, and 75th percentile.

Habitat Assessment. The spawning habitat station in each river will be classified as *Suitable* or *Impaired* based on the performance specification in the Table 3.1. The sources of these designations will be *Mass*DEP Surface Water Quality Criteria (temp., DO, and pH), US EPA's nutrient recommendations for Sub-Region 59 (TN, TP, turbidity and chlorophyll *a*), and BPJ for the physical habitat characteristics. For this SOP version, *Mass*DEP's *Suitable* designation will have equal standing as US EPA's *Minimally Impacted* criteria. The BPJ designations will utilize all available observations and data to assess a classification of *Suitable* or *Impaired* for the physical variables. Any classifications of *Impaired* will result in the documentation of the river reach where spawning habitat is present as *Impaired* with a list of the impaired variables (ex. the smelt spawning habitat in the Stony River is *Impaired* due to pH and DO criteria violations).

Data Management

Chain of Custody. The Field Coordinator will be responsible for collection and processing of nutrient samples from the field to freezer storage at UNH; and will maintain a sample list (Form 3.2) that includes date of collection and date of analysis and will serve as a chain of custody form. Samples will be placed in zip-lock bags and in coolers with ice and driven or shipped overnight to the contract laboratory.

Data Documentation. Specific data forms will be used for each data collection task. Water chemistry and flow data will be recorded on Form 3.1 manually in the field or downloaded directly to an annual water chemistry Excel datafile, depending on the data logging capabilities of field instruments. Nutrient data will be received from the analytical laboratory on Form 3.2 as an electronic file and downloaded to an annual nutrient Excel datafile. Field notes on tile collections will be recorded on Form 3.3. Periphyton biomass data will be manually entered to Form 3.4 and transcribed to an annual periphyton biomass Excel datafile. Periphyton identification data will be entered directly into a periphyton identification Excel datafile. It is recommended that each sampling trip is assigned a common trip label that accounts for state, year and sampling trip (Ex. MA18-01). The sampling trip label will have a two-letter river code and a 1-3 letter code for sample type (ex. MA18-01-FR-TN). A separate column will record the type of sample (sample =1, duplicate = 2, triplicate = 3, blank = B). All quality assurance and control review will be conducted within the individual annual Excel files. When data have been classified and accepted by the QA/QC Analyst the annual files will be combined to a single Excel database. The *Final* datasheet will only contain accepted data for use in subsequent analyses (no *Censored*, replicate, blank, or spike data).

Database Management. Data files will be saved on the common server (W:\) and back-up files will be saved on primary server (P:\) of the Database Manager. The data classification will be updated by the QA/QC Analyst and care should be made to ensure the back-ups are consistent with the primary files. Once all possible review is completed and data has received the *Final* classification, the annual datafile will be saved as read-only files in both servers.

Datafile Classification. The QA/QC Analyst should review the data and classify the QA/QC review status and data status using the classes listed below. The QA/QC status classes refer to the review stage for the entire datafile. The data status classes refer to the status of data under the QA/QC review. This datafile classification is consistent for all four SOPs in this QAPP.

QA/QC Status

1. Draft. Data processing is in progress, and QA/QC has not been conducted.

2. Preliminary. Data processing is complete, but QA/QC is not complete. Data can be used for internal project summaries.

3. Complete. All data processing and QA/QC review is completed.

Data Status

1. Preliminary. Data have been entered from field sheets or downloaded but QA/QC review is not complete.

2. Censored. Data are eliminated because of instrument failure or QA/QC performance.

3. Conditional. Data are fully audited, and QA is complete, but have deficiencies that are documented and may limit use.

4. Final. Data are fully audited, checked and acceptable.

TECHNICAL NOTES

The first four technical notes are not recommendations for optional SOP methods. The topics presented are commonly acknowledged limitations related to periphyton sampling that should be understood by program participants for this SOP and considered for future revisions.

Filamentous Algae. Two-week deployments may not well represent slow-growing filamentous algae. Secondly, cell counts during periphyton identification may not capture the contribution of filamentous algae. This limitation should be acknowledged within program applications. Most MA periphyton communities appear to be diatom dominated during the spring. However, if needed, program participants should consider additional procedures to gain more information on filamentous algae (longer deployments, sample natural substrata, measure algae biovolume, % cover or subsample cell counts of filamentous algae strands) for future projects.

Tile Growth vs. Natural Substrate. Tile growth is beneficial in providing productivity estimates that can help characterize the status of eutrophication in rivers. However, the periphyton growth on tiles will represent first-growth, colonizing cells and may not depict all species that influence smelt egg survival. Similar to filamentous algae, this limitation should be acknowledged within program applications. The methods of ME DEP (Danielson 2006) for sampling natural rocks in wadeable streams has been adopted as a supplement to tile sampling for this SOP. Natural rock samples provide information on the standing algae community but are not controlled samples or measures of growth rates. Future efforts should evaluate the differences in methods and sampling results from these two periphyton sample sources when this SOP is revised.

Percent Cover of Periphyton. Another alternative or supplement to tile sampling is the estimation of percent cover of periphyton on substrata. *Mass*DEP considers a percent macroalgae (ex. green filamentous algae) cover of >50% to indicate degraded habitat and organic enrichment and provide an approach for estimating percent cover in streams (Beskenis 2002). Three samples are recorded at each of three transects crossing riffle habitat. *Mass*DEP is currently developing draft nutrient criteria for streams for aesthetics and aquatic life use using biological indicators, such as benthic algal biomass and % cover of macroalgae.

Species Identification. A large number of methods have been used to identify and enumerate periphyton taxa. Cell counts can easily be applied and provide information on relative percent abundance and dominance. Cell counts alone can over-estimate detrital diatoms and under-estimate the contribution of filamentous algae. Diatom treatments and biovolume estimates are options to improve data quality, but have not been selected because our desired level of taxonomic resolution does not justify the added cost and labor

M^3 Preservative. Add 5 g potassium iodide, 50 ml glacial acetic acid and 250 ml formalin and bring to 1 liter with distilled water. The recommended dose for algal preservation is 2 ml of M^3 per 100 ml of sample. We have used 3 ml of M^3 per sample jar to ensure that samples from tiles with high growth are well preserved. This preservative should be dispensed in a well-ventilated area and kept in laboratory storage designated for acids and preservatives. Dilutions of 3 ml per 100 ml of sample (primarily water) can be discarded down laboratory sink drains.

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Section 4.0 Assessment of River Herring Spawning and Nursery Habitat

SCOPE AND APPLICATION

River herring is the common name used for two anadromous fish, the blueback herring (*Alosa aestivalis*) and the alewife (*Alosa pseudoharengus*) that are similar in appearance and sympatric for most of their range. River herring make spring spawning runs from marine waters into freshwater rivers, lakes and ponds where eggs are deposited and juveniles grow for typically 2-6 months before emigrating to the ocean. River herring use a wide range of habitats for spawning and juvenile rearing across their native range from Florida to Newfoundland (Greene et al. 2009). In New England coastal rivers, river herring spawn in headwater lakes and ponds, as well as main stem rivers. The spawning runs were important sources of commerce in New England coastal towns until the latter half of the 20th century, and recently have been valued as forage to many species of wildlife and by citizens that harvest river herring for food and bait and appreciate the spawning runs as symbols of spring and healthy rivers. River herring populations in Massachusetts have declined in recent years, prompting DMF to ban harvest in 2006.

The causes of river herring population decline are not fully defined and involve both site-specific impairments and range-wide threats at marine and freshwater habitats. The influence of coastal watershed development on the trophic status of ponds and lakes is an overarching theme that connects to emerging concerns over a warming climate, water supply and the rise of invasive plants. These influences may be manifested in increasing symptoms of eutrophication in the form of exacerbated conditions of diurnal DO and pH cycling and summer stratification. Ultimately, these changes could limit water and habitat quality for river herring spawning and nursery requirements.

An important component of river herring population restoration is the assessment of the condition of spawning and nursery habitat in freshwater rivers, lakes and ponds. Section 4.0 outlines the target parameters and techniques needed to assess habitats and to identify water quality and habitat deficiencies. In many cases it is advantageous for local organizations to assist with data collection and restoration efforts. The first step is to consult with DMF fisheries biologists on the status of river herring in a river system based on existing knowledge and previous surveys (Reback et al. 2004a-d). If additional data are needed to confirm the habitat status, the following guidelines can be applied.

The criteria presented in SOP 4.0 were selected to allow low-cost and short-term assessments of water bodies to assist resource management decisions. An important secondary goal of this SOP is to contribute to the Clean Water Act (CWA) processes of develop criteria that can be incorporated into water quality assessments under Section 305(b) (MassDEP 2018) and contribute to assessment, restoration, and protection efforts under Section 303(d) U.S. EPA 2013). The CWA is administered at the Federal level by the US EPA and in Massachusetts by *Mass*DEP and is one of the most significant regulatory processes related to aquatic restoration. Through these processes, the development of water quality criteria associated with river herring life history and habitat requirements could become a valuable tool for both protecting and restoring water bodies and river herring populations.

An important step was made towards this goal with the publication of DEP's CALM (Consolidated Assessment and Listing Methodology) Guidance Manual (MassDEP 2018). The CALM manual describes the methods used to meet CWA reporting requirements under Sections 305 (b) and303 (d). The 2018 manual included Diadromous Fish Habitat assessment guidance that was based on information provided by, (1) SOP 4.0 river herring spawning and nursery habitat assessments; (2) the DMF Diadromous Fish Restoration Priority List; and (3) DMF fish passage surveys. This guidance and the relation to the QAPP update are discussed in greater detail later under Fish Passage Impediments.

Relationships between river herring life history and habitat quality are not well developed. Efforts to establish water quality standards for Pacific salmon habitat had difficulty determining biotic responses to human-induced stressors with adequate reliability and precision (Bauer and Ralph 2001). Habitat suitability indices (HSI) have been developed for shad and river herring with limited success relating the indices to habitat quality or population production (Bilkovic et al. 2002; Kocovsky et al. 2008). Pardue (1983) developed a HSI for river herring that depended on variables for spawning substrate, water temperature, zooplankton abundance, salinity and nursery water temperature. This Section 4.0 update will not attempt to generate a numeric HSI. Section 4.0 will use existing data, scientific literature and field measurements to relate river herring life history to relevant water quality criteria (Mass. SWQS, 314 CMR 4.00, *Mass*DEP 2018; and US EPA 2001) and develop best professional judgment (BPJ) criteria for other important habitat features such as passage barriers, stream flow, and spawning substrate.

Monitoring Objectives

The primary objectives of SOP 4.0 monitoring are to determine if water quality is suitable to support river herring egg incubation and juvenile rearing, and to relate conditions of passage impediments and flow conditions to migratory requirements.

LAKES AND PONDS

Water Quality Monitoring

Sample Stations. Existing information sources should be reviewed to guide decisions on monitoring targets and to assist the design of habitat assessments and selection of sampling stations. The Massachusetts Division of Fish and Wildlife's (*MassWildlife*) bathymetric maps https://www.mass.gov/info-details/massachusetts-pond-maps and regional pond monitoring programs such as the Cape Cod Pond and Lake Atlas (Eichner et al. 2003) and the collaborative PALS sampling program https://www.capecodgroundwater.org/ponds-estuaries/stewardship-program/ are valuable design references. In addition, the MassDEP catalog of water quality assessments should be checked to see if the targeted river system has been previously sampled. http://www.mass.gov/eea/agencies/massdep/water/watersheds/water-quality-assessments.html

The preferred format is to select a shoal station (<2 m depth), a mid-depth (2-7 m) and deep (>7 m) station on a transect line through the center of the pond that connects the pond inlet and outlet. Typically, three sampling stations should be established in each impoundment. Small, shallow ponds (<5 acres) may have just two stations to represent spawning and nursery habitat. Large impoundments (>100 acres) may require additional stations. Project resources and the size, shape and bathymetry of the water body will influence station selection. The surface measurement should target the depth of 0.3 m and the bottom measurement should target 0.5 m off the bottom. Given errors in depth measurements, the range of 0.3 to 1.0 m off the bottom is acceptable. Measurements <0.3 m from the surface or bottom should be avoided because chemistry variability can occur from surface interactions and disrupting bottom sediment. Water column measurements should follow 1-m depth intervals starting at 1.0 m of depth. Consideration can be made at deeper stations (>12 m) to reduce samples by using a 2-m depth interval as long as the sampling allows the characterization of the thermocline.

Sampling Period and Frequency. Water chemistry measurements should be made at the targeted lake or pond during the months when adult spawning and juvenile growth occurs. The period of May-September should be sampled to capture worst case water quality during the spawning and nursery season. Water quality is typically not a concern for May but it is an important month for spawning activity and passage limitations could exist. A monthly sample should be targeted for the second or third week of the month during May-September.

Water Quality Parameters. Basic water chemistry parameters will be compared to *Mass*DEP's SWQS and river herring biological requirements (see Reference Conditions) to determine if the water body is suitable for supporting spawning and juvenile growth. The following parameters should be recorded: water temperature, specific conductivity, pH, dissolved oxygen (DO), turbidity, total nitrogen (TN), total phosphorus (TP), depth, and Secchi disc depth. Water chemistry sampling should follow SOP 2.0 for YSI sondes. If other instruments are used, protocols should be documented in SOP appendices. Refer to SOP 3.0 for methods and QA/QC on TN and TP sampling.

<u>Passage Impediments</u>

River herring depend on adequate upstream passage for spring spawning runs and downstream passage for juveniles migrating to marine waters later in the season. In most MA rivers the spring migration path for adult river herring is documented (Reback et al. 2004a-d) and spring flows are not typically a limiting factor to migration success. The focus for passage assessment for a majority of projects will be the emigration of juveniles in the summer and fall. The onset of juvenile emigration is usually the early summer, although juveniles will exit with much variability until late fall (Kosa and Mather 2001; Yako et al. 2002; Iafrate and Oliveria 2008; Gahagan et al 2010). The point of spawning habitat entry/exit (outlet) should be inspected with each site visit to assess passage potential. The migration path downstream of the outlet should be reviewed (Reback et al. 2004a-d) and surveyed by foot or boat if the reach is unfamiliar to program participants. Obstructions that impede passage downstream of the outlet should be identified and added as sampling stations as necessary.

The following information should be recorded at each Fish Passage station. Water surface width and depth (± 1 cm) should be measured at each station outlet (top fishway weir or structure that acts as hydraulic control). Depth should be recorded at a minimum of three locations (25, 50 and 75% of stream width) at the outlet with additional measurements every meter of channel width for wider channels (> 5 m). Discharge data from a nearby USGS streamflow gage should be recorded if available. The water surface level of the impoundment should be assessed with each visit from an existing gauge or the creation of a new relative staff gage. If needed, a location should be selected next to the outlet on the pond side to measure relative gauge height (± 1 cm) from the water surface. The water depth (± 1 cm) at mid-channel should be measured at a representative minimum depth location in the 10 m reach below the outlet. The minimum water depth recommended by DMF for adult river herring passage is 6 inches (15.2 cm).

In addition to physical measurements, BPJ observations should be recorded on the potential for successful passage (Form 4.1). Fish Passage and Stream Flow observations listed in Form 4.1 are designed to indicate if it is possible for adult river herring to safely migrate upstream to spawning habitat and juvenile river herring safely migrate downstream from nursery habitat. The BPJ classifications are further described in this section under Assessment Criteria.

<u>Spawning Substrate</u>

A wide range of spawning substrate is used by river herring for depositing eggs (Pardue 1983; Bozeman and Van Den Avyle 1989; O'Connell and Angermeier 1997). Fertilized eggs are demersal and adhesive for 24 hours and will stick to any surface encountered. After 24 hours the eggs become non-adhesive and hatching typically occurs within 3-4 days. Depending on the river system, there can be spatial overlap or isolation in spawning habitat use for the two species. Although preferences are not well documented, in New England coastal streams, alewife appear to target shallow fringes of headwater ponds where coarse sediment and gravel may be more suitable for egg incubation than fine sediments or dense periphyton. The percentage of substrate type should be visually estimated to the nearest 10% at each shoal station. Substrate observations can extend beyond the immediate station as needed to determine a representative percentage of bottom cover. A long-handled, D-shaped aquatic net is recommended for raising substrate samples from the bottom. Percentages should be assigned on Form 4.1 for the following substrate types: silt (<0.06 mm diameter), sand (0.06-2.0 mm), gravel (2-64 mm), cobble (64-256 mm), boulder (>256 mm), detritus, periphyton, aquatic moss, and vascular plants. The assignments should be made for the substrate surface. In some cases, the sub-surface material is 100% silt or sand. This condition should be noted; however, monthly assignments should reflect changes in plant growth on the substrata.

Because of the variety of spawning substrata used by river herring and the lack of consensus in the literature over optimal habitat, no substrate criterion was selected for this QAPP version. Observations on the influence of beach nourishment, water supply management, and streambank erosion should be recorded.

Lakes and Ponds (Optional). If necessary and project resources are available, quantitative data can be obtained on spawning substrate. A 50 m transect can be set parallel to shore at shoal stations where six random, grab samples can be collected along the transect. The transect location should represent the typical substrate type along the shore next to the shoal station and will target 1-2 m of depth in most cases. A small bottom dredge should be used that collects approximately 100-200 cm^2 of material. The collected sediments can be measured following Wentworth's classification of sediments (Nielson and Johnson 1983) and all substrata types can be assigned a percentage of volumetric measurements. This includes macrophytes and periphyton identified to the lowest possible taxa and classified as native or invasive.

Native and Invasive Plants. Vascular plants and macroalgae should be identified at shoal stations with each sampling visit to the lowest possible taxonomic level. In many cases, identification to the genus will be suitable. The exception is for invasive plants where species identification is recommended. Two plant identification guides produced by the New England Aquarium (Kelly 1999) and Massachusetts Department of Conservation and Recreation (DCR 2010) for Massachusetts ponds are recommended. Assign a qualitative rank for biomass for each identified plant during each shoal station visit: (1) most abundant (record as *dominant* if >75% of plant biomass) (2) 2nd most abundant, (3) 3rd most abundant, (4) common and (5) trace. Photographs should be taken of invasive plants and to aid identification. Habitat assessment reporting should include a brief summary on the plant community observed.

Velocity and Discharge Measurements. Stream flow data should be recorded if gages are located close to sampling stations in order to relate discharge to water depth. In the absence of gage stations, consideration should be given to measuring water velocity and discharge at the outlet transect. Measuring velocity at the outlet station will be useful in cases where a suspected velocity barrier exists or swift flow is present at a fishway entrance. Water velocity at outlet stations should be measured at the same transects and locations as depth measurements. The current meter should be positioned at six-tenths of the water depth. Do not use automatic readings for instantaneous measurements of flow; instead record average velocity over a 40 second interval. Discharge measurements should follow the USGS midsection method described in Buchanan and Somers (1969). See SOP 3.0 for instructions and QA/QC for discharge measurements.

Sampling Frequency. It is acknowledged that the SOP 4.0 sampling frequency produces relatively low spatial and temporal coverage. If resources are available, consideration can be given to increasing the sampling frequency to two samples per month or deploying multi-probe water quality sondes to continuously log data. These instruments are costly and require intensive QA/QC review (see SOP 2.0). However, extended deployments during the warmest period of summer will better characterize water quality than five grab samples. Deploying these instruments at shoal stations will also capture daily DO cycles and the influence of stormwater events. Sampling in the fringe months of April and October can be considered if additional site-specific information is needed on spring or fall migration, spawning or nursery habitat conditions.

Random Station Sampling. The adoption of fixed stations along a pond transect was made to allow easily executed, repeated measurements at the same locations. This suited the interest of classifying river herring habitat status in waterbodies with limited previous monitoring and high spatial and temporal variability. However, fixed stations limit investigations of sampling bias and on statistical inferences across space and time (Bonar et al. 2009). Random stratified sampling is a preferred design approach if hypothesis testing and/or site and year comparisons are desired. Random sampling can be considered for cases where projects seek more information than provided by typical two-year assessments. Pond locations should be divided into strata for shallow, mid-depth and deep water depth. A minimum of three shallow, one mid-depth and one deep station should be drawn for each separate sampling trip. It is presently recommended that this approach is done concurrently with an established fixed station transect. This approach will be instructive for future practices, although not suitable for all projects due to the added complexity of sampling and practical concerns over the feasibility of longer work-days and extending temporal biases in chemistry data.

Pond Morphometric Data. The morphology of a ponds or lake is intrinsically related to their water quality and physical and biological characteristics (Wetzel 1983). Our river herring habitat assessments to date have demonstrated widespread impairments relative to the adopted thresholds and raised concerns over the extent of habitat loss due to seasonal stratification. However, the results add little to the knowledge of pond morphology and possible relations to water quality impairment. Assessments conducted under the updated QAPP should strive to record more information that can be used to consider causal factors for impairments and the concept of fish productivity or carrying capacity (Milner et al. 1985; Budy et al. 2009).

In order to gain more information on possible relationships to pond morphology and river herring habitat suitability and carrying capacity, the following pond metrics should be recorded under QAPP V2 habitat assessments:
- Pond volume, area, perimeter
- Maximum depth
- Mean depth
- Ratio of mean to maximum depth
- Mean ratio of light penetration (Secchi disk depth) to maximum depth
- Mean ratio of anoxic zone to maximum depth
- Water volume (will require previous citations)
- Epilimnion and hypoliminion water volume

Food Supply. Juvenile river herring feed on a variety of aquatic invertebrates, including copepods, dipterian midges, and cladocerns (Pardue 1983). Although food supply is vital for nursery habitat, in most cases, zooplankton sampling is beyond the scope of the SOP. If resources and project interests allow an investigation of pond food supply then the project staff should review suitable literature starting with Rabeni (1996).

Temperature Loggers. Continuous temperature loggers are a useful option in ponds that have warm water approaching the water temperature criterion and for assessing the 7-day mean of daily maximum temperature. Temperature loggers can also provide data on fish migration influences. See SOP 1.0 for logger deployment instructions. Site selection in lakes and ponds will take careful consideration to account for inlets/outlets, and depth variation.

RIVERS

A large majority of cases where spawning habitat assessments are needed will involve lentic habitat in lakes and ponds. In some Massachusetts river systems, particularly with substantial passage alterations, there appears to be little spatial segregation in spawning habitat use by the two species. However, there is a general understanding that while both species tend to spawn in lakes and ponds, blueback herring will also occupy lotic spawning habitat (Loesch and Lund 1977; Pardue 1983; Bozeman and Van Den Avyle 1989; Collette and Klein-MacPhee 2002). Some assessments may be needed where river herring spawn in the lotic flow of river channels. The monitoring objectives to assess water quality, passage impediments, and substrate in rivers are the same as with lakes and ponds. However, sample station selection and depth measurements will differ and require a case-by-case evaluation that is supplemented by reviewing existing knowledge and data on the river. River assessments should use SOP 2.0-4.0 guidance and describe supplemental methods in the resulting assessment reports.

ASSESSMENT CRITERIA

The objective of assessing the suitability of river herring spawning and nursery habitat will be met by comparing monitoring data to quantitative criteria for water temperature, pH, DO, Secchi disk, TN, and TP; and qualitative criteria on eutrophication, passage barriers, and stream flow. The assessment criteria (Table 4.1) are derived from a synthesis of the available scientific literature, MassDEP's Surface Water Quality Standards (SWQS) (MassDEP 2018), US EPA nutrient criteria (US EPA 2000c) and BPJ (see Greene et al. 2009). For most criteria, existing knowledge is insufficient to clearly establish thresholds for both blueback herring and alewife survival at all critical life stages. Such thresholds have been assessed for anadromous striped bass (*Morone saxatillis*) (Hall 1991) and may be adopted in later versions of SOP 4.0 as information becomes available.

Classification: Reference Conditions

Nutrients. The US EPA's Nutrient Criteria Technical Guidance Manual for Lakes and Reservoirs (US EPA 2000) recommends several statistical approaches for developing nutrient criteria for total phosphorus (TP), total nitrogen (TN), chlorophyll *a* (chl *a*), and Secchi disk. In the absence of data on minimally impacted (reference) conditions for protecting designated uses, US EPA recommends using the lower 25th percentile of the distribution of measured variables from a population of lakes and ponds within a region. The 25th percentile serves as a threshold between minimally impacted and degraded locations. The US EPA has generated reference conditions using the median of the four seasonal 25th percentiles for all lakes and ponds sampled in the Northeastern Coastal Zone (Ecoregion 14, sub-region 59; US EPA 2001). This SOP adopts EPA's nutrient criteria recommendations for Ecoregion 14 to assess the influence of eutrophication on water quality (Table 4.1). In addition, independent reference conditions will be calculated using field data from all ponds (25th percentile) once an adequate number of Section 4.0 assessments have been conducted. These data can also contribute to the development of designated use criteria related to river herring spawning and nursery habitat.

Physico-Chemical. The US EPA recommendations for nutrient criteria do not include criteria for water chemistry response variables such as DO and pH. For this QAPP version, thresholds for habitat classifications will be adopted using the scientific literature on river herring and guidelines from MassDEP's SWQS on temperature, DO, and pH (Class B Warm Water Fishery). These thresholds along with other variables related to migratory, spawning, and nursery habitat may be refined in future versions as the state of knowledge on this topic improves. Reference criteria are presented in Table 4.1 and discussed in the following paragraphs.

MassDEP's approach to setting SWQC continues to evolve with improvements in scientific knowledge and supporting technologies. Recent updates (MassDEP 2018) have relied less on strict parameter thresholds and more on integrated approaches that consider exposure and weight-of-evidence (multiple indicators) concepts and the relation of water quality to natural background conditions. These approaches generally require more information on water quality data and additional steps of BPJ by data reviewers. No changes are proposed to the assessment sampling frequency for this QAPP update (N = 10; two years of sampling of May-September). Therefore, major updates to this QAPP's data classification process will not be made. However, the above themes will be integrated into the parameter classification process where practical and discussed below.

Table 4.1 Physical, Chemical and Biotic Criteria used for Reference Conditions and Best Professional Judgment Classifications at River Herring Spawning and Nursery Habitat.

Variables	**Suitable** (SWQC or BPJ)	**Minimally Impacted** (25th percentile)	**Notes/Source**
REFERENCE			
Temperature (ºC) (July-Oct.-nursery)	≤ 28.3		Maximum limit (MassDEP 2007)
Temperature (ºC) (May-June -spawning)	≤ 26.0		Scientific literature and BPJ
Temperature (ºC) (May-June spawning)	≤ 20.0 (7-day mean)		7-day mean of daily max. from logger data (*Mass*DEP 2007)
pH	≥ 6.5 to ≤ 8.3		(MassDEP 2007)
DO (mg/L)	≥ 5.0		(MassDEP 2007; see page 51 for *DO Deep Water* criterion)
Secchi disc (m)		≥ 2.0	75th percentile; EPA Ecoregion 14, sub-84 (US EPA 2000)
Turbidity (NTU)		≤ 1.7 (rivers only)	EPA Ecoregion 14, sub-59 (US EPA 2000)
TN (mg/L)		≤ 0.32	EPA Ecoregion 14, sub-59 (US EPA 2000)
TP (*u*g/L)		≤ 8.0	EPA Ecoregion 14, sub-59 (US EPA 2000)
Chlorophyll a (ug/L) (Fluorometric)		≤ 4.2	EPA Ecoregion 14, sub-59 (US EPA 2000)
QUALITATIVE			
Fish Passage	BPJ		SOP Section 4.0
Stream Flow	BPJ		SOP Section 4.0
Eutrophication	BPJ		SOP Section 4.0

Water Temperature. Studies on critical temperatures for river herring have produced variable results and do not fully describe all early life history concerns (Greene et al. 2009). Optimal spawning temperatures were assumed to be 15-20 ºC for alewife and 20-24 ºC for blueback herring (Pardue 1983). Kellogg (1982) reported that hatching success for alewife eggs declines sharply at 26.7-26.8 ºC and that larval and juvenile survival is supported at higher temperatures. Alewife temperature preferences have been reported as 26.3 ºC for larvae

(Kellogg 1982) and 19-25 °C for juveniles (Otto et al. 1976). A more recent study on the survival of embryonic alewife (24 hours post-fertilization) found maximum survival of alewife eggs occurred from 13-15 °C and that mortality increased significantly above 18 °C (O'Keefe and Skomal 2005).

The application of water temperature criteria for river herring is difficult because four life history stages of two species occur during a wide range of temperatures. For example, Kellogg's (1982) optimal temperature for alewife larvae growth is >10 ºC warmer than peak spawning periods. This SOP continues to use the three temperature criteria adopted in QAPP V1 to account for different life stages and the uncertain status of the available information on this topic. The Massachusetts SWQS water temperature criterion (Class B) of ≤28.3 ºC for support of aquatic life in warm water fisheries will be used for the nursery period of July-October. The cold water fishery SWQS of ≤20 ºC for the 7-day mean of daily maxima will be used for the spawning months of May and June when temperature logger data are available. Lastly, 26.0 ºC was identified as an upper threshold for suitable temperature ranges for alewife egg hatching (Kellogg 1982) and for blueback herring prolarva (Klauda et al. 1991). Based on these scientific citations and a review by Greene et al. (2009), ≤26.0 ºC is considered *Suitable* for river herring early life history during May-June.

A review of past habitat assessments did reveal that deeper waterbodies with numerous measurements in the cooler hypolimnion were less likely to reach the exceedance threshold of 10% of samples than shallow ponds. Therefore, to reduce this bias the temperature classification will only be derived from measurements made in the upper 0 – 5 m of the water column.

Water pH. The acidification of surface waters is a recognized ecological concern for aquatic resources and fish populations (Haines 1981; Haines and Johnson 1982). Environmental acidification has been linked to the elimination of anadromous populations and chronic poor recruitment of anadromous fish in North America. The disruption of ionoregulation in gill tissues is a primary cause of death related to low pH levels. Studies on blueback herring from Chesapeake Bay tributaries report survival data that contribute to the setting of pH thresholds (Klauda and Palmer 1985; Klauda et al. 1987). Fertilized blueback eggs were more tolerant of acidity than yolk-sac larvae and had the following mortality rates during static pH treatments with no aluminum: 69% at 5.0 pH, 7% at 5.7 pH, 7% at 6.5 pH, and 6% at 7.8 pH. The same treatment for yolk-sac larvae resulted in the following mortality rates: 99% at 5.0 pH, 89% at 5.7 pH, 38% at 6.5 pH, and 16% at 7.8 pH. Mortality increased with higher concentrations of aluminum and increasing duration of exposure. The overall trend for yolk-sac larvae was rapidly improving survival at ≥6.5 pH and declining survival below 6.5 pH. Conversely, high pH values (alkaline) can be related to ammonia toxicity in aquatic life. The SWQS for pH in Class B waters is within the range of 6.5 – 8.3 pH. The SWQS pH range is adopted as *Suitable* for river herring spawning and nursery habitat. Values outside the range will be assessed as *Impaired*.

Photosynthesis and respiration are major influences on water pH with distinct diurnal cycles. Related, stratified lakes in the summer will display a vertical distribution of high pH in surface waters and declining pH at depth. In eutrophic lakes the vertical curve in pH can be pronounced with low pH in the hypolimnion and higher pH at the surface that increases in the afternoon. QAPP V1 used all pH measurements to classify a water body; often resulting in a high percentage of samples <6.5 pH in lakes with deep stations. To reduce the influence of low natural pH in deeper waters, QAPP V2 will retain QAPP V1 pH thresholds but derive the classification only from measurements made in the upper 0 – 5 m of the water column.

The adverse effect on fish health of increasing hydrogen ions can be augmented by the mobilization of metal ions (Haines 1981). Increasing aluminum concentrations will increase fish egg and larvae mortality in low pH water. Klauda and Palmer (1985) also demonstrated higher tolerance of blueback eggs and larvae to episodic exposure to low pH and rapidly increasing mortality when exposure duration exceeded 24 hours. In most cases, the analysis of metals in surface waters and continuous pH measurements will be beyond the SOP scope. In addition to taking discrete pH measurements, available information for each water body should be reviewed for data trends in pH and metal ion concentrations. Further, aluminum treatments of ponds are increasing in MA to reduce the eutrophic effects of phosphorus loading (Wagner et al. 2017). Applications of aluminum sulfate and sodium aluminate are used to bind phosphorus and can temporarily depress pH. Habitat assessments should review the monitoring of present and past aluminum treatments at sample locations and document the status during reporting.

Dissolved Oxygen. Dissolved oxygen concentrations in water are highly influenced by temperature and biological processes, resulting in seasonal and diurnal cycles. Eutrophied water bodies can display DO fluctuations that become a threat to aquatic life. Plants produce DO during daylight photosynthesis. At night, they consume DO and produce carbon dioxide. Therefore, the lowest DO occurs just before sunrise and supersaturation can occur later in the day. Critical swings in DO can occur during the warmest summer days when high algal growth reduces DO at night to low levels that may remain suppressed during cloudy days. Seasonal and daily depression of DO is a major concern for the health of nursery habitat; as severe conditions can limit habitat availability, or in worst cases, cause widespread fish mortality.

The specific tolerances of early life stages of river herring to DO extremes and fluctuations are not well described. Water temperature is critically linked to the influence of DO on river herring survival. Rising temperature reduces the capacity of water to maintain DO concentrations. Jones et al. (1978) offered a recommendation a minimum DO concentration of 5.0 mg/L for eggs, larvae and adult for alewife and blueback. Bozeman and Van Den Avyle (1989) exposed juvenile blueback herring to hypoxic conditions and reported mass mortalities at 3.6 mg/L DO and 27.6 °C; however, limited survival was observed with short-term exposure <3.0 mg/L. A habitat requirement of 5.0 mg/L was adopted for striped bass larvae and juveniles following findings that egg survival could occur with DO <5.0 mg/L; although, the incidence of deformed larvae and egg mortality increased with hatching below 4.0 mg/L (Hall 1991).

The Massachusetts SWQS for DO in Class SB waters of ≥5.0 mg/L was adopted as *Suitable* for QAPP V1. This threshold will be used again in QAPP V2, although the DO classification will be modified to include metrics for both surface water and deep water impairment.

Dissolved oxygen concentrations in a given waterbody can vary substantially due to changes in temperature, precipitation and wind direction. Changes in environmental conditions can diminish the capability of monthly monitoring to assess the suitability of DO concentrations for supporting aquatic life. Another consideration is the effect of naturally occurring thermal stratification (Wetzel 1983). Deeper lakes and ponds typically begin to stratify during early summer. The upper layer (epilimnion) becomes separated from the lower layer (hypolimnion) by a thermocline often near 4-6 m. The epilimnion continues to warm as summer progresses and remains oxygenated due to surface disruption and photosynthesis. The hypolimnion becomes hypoxic or anoxic as bacteria in bottom sediments consume oxygen. This zone can become poor habitat for fish, including alewife (Lindenberg 1976), until the stratification breaks down with increased wind in autumn. The presence of hypolimnetic anoxia is problematic for DO classifications because it is a natural and common occurrence in large, productive lakes.

Given the natural presence of hypolimnetic DO depletion, QAPP V1 excluded DO bottom measurements at deep and mid-depth stations from DO classifications. Habitats were classified as *Impaired* when >10% of DO measurements were <5.0 mg/L - exclusive of the bottom measurements. The experience of our assessments under QAPP V1 has shown that the persistence of hypolimentic DO depletion higher in the water column caused most deeper lakes to be classified as *Impaired* for DO whether or not the conditions were caused by natural stratification. For QAPP V2 we are integrating MassDEP’s weight-of-evidence approach for DO and will increase the exemption of hypolimnion measurements to avoid what may have been an overly sensitive DO classification.

Mass*DEP DO Criterion.* MassDEP has enhanced its approach to DO classification under SWQC to use the exposure concept (frequency, magnitude, and duration) with a reliance on continuous DO sonde deployments (MassDEP 2018). Further, MassDEP is integrating weight-of-evidence indicators to further refine the classification process. These include the % of watershed with natural wetlands, DO depletion from natural conditions in relation to lake surface area, DO supersaturation, and diel changes in DO concentrations (MassDEP 2018). Given the high data requirements to acquire some of these indicators, QAPP V2 will retain the DO threshold of 5.0 mg/L, while adopting the MassDEP guidelines for DO saturation and diurnal change to enhance our BPJ Eutrophication classification and introducing new metrics related to the extent of the hypolimnion.

DO - Surface Waters. The 5.0 mg/L DO threshold will be retained in QAPP V2 but will apply to all measurements recorded at transect stations during May-September at depths 0-5 m. The classification will continue to allow a 10% exceedance <5.0 mg/L, with a bottom measurement exemption for mid-depth stations (at 2-7 m depth). A *Suitable* classification will be made if ≤10% of the measurements are <5.0 mg/L. An *Impaired* classification will be made is >10% of the measurements are <5.0 mg/L.

DO – Deep Waters. A second DO classification will be made for lakes and ponds that have a deep water station (>7 m) subject to seasonal stratification. By sampling at 1-m intervals, the extent of the epiliminion, metalimnion, and hypolimnion will be identified. If hypoxic (<4.0 mg/L) conditions exceed 50% of the water column the water body will be classified as *Impaired* for hypolimnetic DO depletion.

Secchi Disk. Secchi disk is an easily retrieved measurement of water clarity and indicator of water quality that has been widely applied for decades. The measurement is most influenced by suspended plankton and inorganic particles. Yako et al. (2002) and Kosa and Mather (2001) suggest that in small coastal systems, low visibility is a cue for emigration and may affect the foraging ability of juvenile river herring. Of the parameters that presently have US EPA recommended criteria, only Secchi disk is set to the 75^{th} percentile of data distributions. This is because Secchi disk depth (SD) measurements increase with greater water clarity. The US EPA Secchi disk criterion for subecoregion 59 is 4.9 m, representing the 75^{th} percentile of sampled lakes for all four seasons. This high water clarity is not likely for many Massachusetts lakes and ponds during May-September. The SD criterion for subecoregion 84 (including Cape Cod) is 2.0 m; a value that represents a more likely threshold for degraded water quality. MassDEP has a SWQS threshold for SD of 1.2 m under the designated use of Primary Contact Recreation. Until river herring assessment data can be accumulated to develop an independent reference for SD, the subecoregion 84 criterion will be adopted for this SOP, and the subecoregion 59 will be used as a secondary threshold of high water clarity.

Carlson Trophic State Index. The Carlson Trophic State Index (TSI) (Carlson 1977; Carlson and Simpson 1996) is a commonly used classification that relates water chemistry indicators associated with algal biomass to an expected range of trophic conditions. The TSI established relationships for TP, chlorophyll a, and SD with a score ranging 0-100. Scores near zero would indicate severe nutrient poor and low productivity conditions, while scores near 100 indicate extremely degraded and highly productive conditions. The TSIs for these parameters relates to a numeric scale of trophic state where the expected changes in trophic status are connected by the concept that increasing nutrients elevate plant productivity and result in reduced water clarity.

Each habitat assessment should generate TSI from averaged TP and SD data using the following equations. Water quality classifications for TP and SD will be based on the EPA reference thresholds and not TSI. However, the results should be compared to trophic status adopted in US EPA (2000) and other suitable references (Eichner et al. 2003) and discussed in assessment reports.

TSI(SD) = 60 – 14.41(ln(SD))
TSI (TP) = (14.42(ln(TP)) +4.15

TSI >50: Eutrophic (high nutrient enrichment and anoxic hypolimnia)

TSI 40-50: Mesotrophic (moderate enrichment)

TSI <40: approaching oligotrophic (low enrichment)

Classification: Best Professional Judgment

Eutrophication. Eutrophication is the response that a waterbody undergoes as it moves towards a highly productive trophic state in the presences of excessive nutrients. Relationships between causal factors of eutrophication and biotic responses are not well defined. Consequently, widely accepted quantitative criteria on eutrophic thresholds are not available. A detailed analysis of the trophic state of freshwater habitats is beyond the scope of this QAPP. Instead, SOP 4.0 assessments will continue to use the US EPA Ecoregion recommendations for TN and TP, and adopt nutrient enrichment indicators from MassDEP's CALM guidelines (MassDEP 2018) to record symptoms of eutrophication Presently, the Massachusetts SWQS do not contain nutrient criteria, although it encourages the development of site-specific criteria.

Common symptoms of chronic nutrient enrichment include reduced water column DO, supersaturated surface DO, reduced water clarity, and increased phytoplankton, periphyton and macroalgae growth. Severe eutrophic conditions can cause fish kills and alterations in natural communities of flora and fauna. A BPJ assessment will be made with each site visit using a combination of measured reference conditions (low DO, high pH, high turbidity, and low SD) and the following MassDEP nutrient enrichment indicators:

- ≥125% DO supersaturation
- Diurnal shift in DO >3 mg/L (cont. loggers only)
- Aquatic macrophyte (non-rooted) coverage >25% of water surface
- Dense biovolume (>50-75%) of all aquatic macrophytes found at >25% of waterbody

Plant growth on substrata and water column will be assigned a percent coverage to the nearest 5%. Any combination of three reference condition exceedances and nutrient enrichment indicators will result in an *Impaired* classification for Eutrophication BPJ. Habitat assessments with <3 such violations will be classified as *Suitable*. This eutrophication classification will be used as a supplement to the nutrient reference condition thresholds to evaluate water quality impairments.

Passage Impediments. With each habitat assessment trip, the condition of the waterbody outlet and any downstream barriers should be recorded. The physical dimensions of flow over the outlet should be recorded on Form 4.1. Field staff should classify the outlet type (dam, culvert, natural, fishway, flume, sluiceway, other) and record the presence (Yes/No) of impediments to upstream or downstream passage. If "Yes" is recorded, then the type of impediment should be recorded in Form 4.1 from the list below.

1. Excess vertical rise or grade change
2. Excess water velocity
3. High turbulence or irregular flow
4. Low or no flow (via stream flow)
5. Low or no flow (diversion /operational)
6. Inadequate attraction flow for passage
7. Shallow water depth for passage (<6")
8. Sediment accumulation
9 Debris blocking passage
10. Beaver dam blocking passage
11. Vegetation blocking passage
12. Degraded passage structure
13. Poor passage structure design

Using the observed conditions above and BPJ, the capacity of an outlet or impediment to provide upstream (adult river herring) and downstream (passive emigration of adults and juveniles) passage should assessed as *impaired*, *suitable*, *optimal* or *unsuitable* for each assessment trip.

The basis for *optimal* (ample flow and no barriers or impediments) and *unsuitable* (passage is not possible) classifications will be readily apparent. The separation of *impaired* and *suitable* can be subtle and requires fish passage experience. This BPJ classification also uses the USFWS definitions for "safe, timely, and effective" fish passage (USFWS 2017). Observed conditions that violate any of the three USFWS definitions below would result in an *impaired* classification. An example of the BPJ classification for *impaired* fish passage would be cases where upstream migration is limited by high fishway entrance velocity or a shallow, craggy channel substrate that does not prevent passage, but significantly delays passage and causes physical damage to adult herring (scale loss). The same would apply for cases when low flow and absence of plunge pool causes mortality to some but not all emigrating juveniles. For most habitat assessments, DMF staff should be providing the monthly assessments or at least provide guidance to program participants to review the impediments and possible classifications.

USFWS (2017) Fish Passage Definitions

Safe Passage – no observations of conditions that could cause injury, death, excess stress or increased predation.

Timely Passage – no observations of conditions that would cause a significant delay in migrations.

Effective Passage – observations of physical and environmental conditions that would allow successful movement through the zone of passage.

The recording of impediment types and passage limitations will also directly contribute to DMF's Diadromous Fish Restoration Priority List, in which the status of passage at all fishways is recorded and restoration potential is ranked. Further, the assigned values for Fish Passage in the Priority List have been adopted by MassDEP for their Aquatic Life assessments under the updated CALM guidance (MassDEP 2018).

Water Velocity

Specific consideration is needed for water velocity because of the complexity of its assessment and its role in both Fish Passage and Stream Flow classifications. The impact of water velocity on fish passage requires species-specific information on swimming speed, endurance and body length, and site specific physical information that can create spatial and temporal variability in water velocity (Castro-Santos 2002; USFWS 2017). Because of these influences, setting discrete thresholds for water velocity barriers is difficult. Further, not all habitat assessment efforts will have the capacity to measure in-stream water velocity.

Swimming performance studies by Castros-Santos (2002) and Haro et al. (2004) provided assessments of the declining ability of blueback herring and alewife to successfully pass velocity barriers over short distances. They reported a 50% passage rate for alewife over a distance of 10 m at 2 m/s (6.56 ft/s) and 0% passage to 25 m. Blueback herring fared slightly better, with a 70% passage rate for a distance of 10 m at 2 m/s and 20% passage rate to 30 m. Water velocity of 3 m/s (9.84 ft/s) resulted in 0% passage for alewife and 10% passage for blueback herring to 10 m. For this QAPP update, a water velocity warning limit of 2 m/s will be used when measurements are available to assist BPJ classifications for Fish Passage (Passage Impediments #2) and Stream Flow.

Stream Flow. Decreased stream flow can reduce the quality and quantity of migratory, spawning and nursery habitat. Juvenile growth can be impaired through negative influences on food sources and mortality can increase through predation and entrapment in dewatered reaches during emigration. In many cases, the assessment of stream flow will be linked with passage impediments because low flow prevents passage over an obstruction. A separate criterion for stream flow is needed for the cases when habitat impairment or suitability resulting from stream flow is independent of an obstruction.

Additionally, documented observations will be useful for cases where stream flow would be ample to support habitat requirements in the absence of an obstruction. Stream flow at each outlet and impediment should be assessed to record if flow supports upstream and downstream passage (Yes/No) on Form 4.1. If "Yes" is recorded, then the type of impediment should be recorded from the list below.

1. Shallow depth: <6 in - adult river herring
2. Shallow depth: <3 in - juvenile river herring
3. Channel braiding and/or dewatering
4. Velocity barrier through zone of passage

Best Professional Judgement should be used to assign one of the following designations for the influence of stream flow on spawning, nursery, and migratory habitat: *impaired*, *suitable*, and *unsuitable* (no flow to support passage). Higher flows that contribute to velocity barriers will not always be classified as *impaired* for Stream Flow; however, the structure that causes the condition is likely to be flagged as *impaired* under Fish Passage.

Assessment Reporting

River herring spawning and nursery habitat assessment data will be processed into datafiles by DMF staff following two seasons of monitoring. The datafiles will be finalized and submitted to project partners and MassDEP. On a case-by-case basis and depending on staff availability, assessment reports summarizing the site data will be drafted for DMF Technical Report series and posted on the website: http://www.mass.gov/eea/agencies/dfg/dmf/publications/technical.html.

Classification Guidance

The following guidance shall be applied for habitat classifications. Final classifications will be assigned for the six reference parameters (water temperature, pH, DO, TN, TP, and Secchi disk), and three BPJ classifications (Eutrophication, Fish Passage and Stream Flow). For example, a waterbody can be classified as *suitable* for DO and *impaired* for Fish Passage. MassDEP allows the classification of *support* for Aquatic Life Uses when infrequent excursions occur for some parameters. In certain cases, MassDEP allows 10% of the representative samples to exceed water quality criteria. A similar approach will be adopted for this SOP. If ≤10% (or ≤1 exceedance for small sample sizes, N = 6-9) of the respective samples at the primary transect stations exceed the MassDEP criteria for the six reference parameters a *suitable* classification will be applied. Exceedances >10% (or >1 exceedance for small sample sizes, N = 6-9) for May-September sampling will trigger an *impaired* classification. The same 10% exceedance rule applies to BPJ classifications. In addition, Fish Passage and Stream Flow can be assigned an *unsuitable* classification if ≥50% of trip observations are *unsuitable*.

Equipment List

1. Multi-probe water quality instrument (see SOP 2.0 for YSI 6-Series Multi-Probe Instruments)
2. Secchi disk
3. Measuring tape
4. Meter stick
5. Gravel scoop attached to broom handle or telescoping pole for sediment grab
6. Handheld GPS unit
7. Kayak, canoe or skiff with anchor and life vests for each passenger
8. Water current meter: either Pygmy style for low flow and depth or Price style for flows >0.25 cfs and depths >0.5 ft
9. Smart phone/camera (all passage structures and outlets should be photographed)

QUALITY ASSURANCE AND CONTROL

Quality assurance and control (QA/QC) protocols will be applied for basic water chemistry, water nutrient samples, and stream flow data collections. The QA/QC review will depend on performance criteria that target indicators of accuracy and precision. The analysis of pre- and post-deployment calibration data will evaluate accuracy for basic water chemistry measurements. Precision will be evaluated by the analysis of the similarity of replicates (field samples, laboratory, and blanks) for water chemistry data. Each data collection will also be subject to an outlier review.

Data Quality Objectives

Data quality objectives are specified for each water quality parameter (Table 1.1) and evaluated primarily through analysis of data accuracy and precision. Water quality data within the accuracy range specified by YSI for each parameter should be attainable with accurate and consistent calibrations. Refer to Table 2.1 in SOP 2.0 for parameter specifications on resolution, range and accuracy. The data quality objectives should be monitored by conducting and reviewing pre-deployment and post-deployment calibrations. The precision of sensor measurements shall be monitored in the field and during laboratory calibrations by recording the relative percent difference [RPD = (difference of two consecutive readings/average of two consecutive readings) x100]. Data quality objectives for the BPJ classifications of Eutrophication, Stream Flow, and Fish Passage are undefined because of the qualitative basis for these criteria.

Water Chemistry

Basic Water Chemistry. All instrument handling, calibration, and calibration data review procedures are outlined in SOP 2.0. Only procedures specific to collecting water chemistry samples for SOP 4.0 are listed here. One duplicate sample will be collected at the surface from one transect station at each waterbody during each sampling trip. The RPD will be calculated from the duplicate and will serve as the field precision measurement for that sampling trip. The sonde will be positioned at the surface (depth sensor reading of 0.3 m) for the duplicate sample and allowed to acclimate for 5 minutes. A measurement will be saved as the sample, followed by the second measurement after two minutes. The second measurement will be used only to generate the RPD precision check.

All duplicates that have an RPD <5% will be accepted. A seasonal mean will be calculated for all parameter measurements (including precision duplicates with RPD < 5%). A warning limit of ±3 SD from the seasonal mean will be used to flag potential outliers. Samples that exceed the seasonal mean by ±3 SD and duplicates with RPD ≥5% will be classified as *Conditional* data and reviewed graphically as outliers and related to sensor calibration performance. Identified outliers that cannot be explained by natural causes should be *Censored.*

Turbidity Exception. Turbidity measure-ments are subject to interference from suspended objects and will show natural variation in stream flow. Also, base flows often have low NTU values, yet minor differences of low values can cause high RPDs. This is a function of proportional statistics and not necessarily related to precision. Therefore, turbidity quality control will follow different warning and control criteria. The warning criterion for turbidity is raised to 25% RPD. Duplicates with RPD ≥25% will be classified as *Conditional* data and reviewed as outliers. Further, precision measurements can easily exceed 25% RPD with very low turbidity values. For example, measurements of 0.2 and 0.3 NTU will yield an RPD of 40%. Therefore, an RPD limit will be relaxed for absolute differences of up to 0.5 NTU for precision measurements ≤ 2.0 NTU.

Depth Measurements. Depth is the only YSI parameter that can be calibrated in the field. The depth sensor can be calibrated by positioning the sonde at the water's surface and entering a calibration value of 0.0 m. Surface measurements should target 0.3 m of depth by positioning the top of the sonde (cable attachment port) at the water's surface. Bottom measurements should be approximately 0.5 m from the bottom. Using cable tension, the user should briefly encounter the bottom and record the bottom depth. The sonde should next be raised up 0.5 m to avoid suspension of bottom sediments. Monitoring turbidity during the 10-minute acclimation period will confirm independence from the bottom.

Stream Flow Data. The Teledyne-Gurley Price current meters used by DMF do not allow user calibration. An accuracy check shall be conducted each week to be sure the bucket wheel is operating according to manufacturer's specifications. The bucket wheel must spin freely without vibration for at least 1.75 minutes. If the current meter fails two consecutive spin checks, it should be serviced as instructed in the operation manual prior to field use. Other types of current meters used by project partners should be documented in a QAPP appendix and tested weekly according to manufacturer specifications to confirm proper performance. Similarly, precision checks are difficult for a single current meter. This is because true differences in water velocity can be expected over small spatial and temporal scales in turbulent riffles. At each flow transect station a single duplicate measurement should be made at one flow cell. All duplicates with RPD <10% will be accepted. Values that exceed 10% RPD will be classified as *Conditional* with no further action other than diligent maintenance and spin checks.

Project and Data Management

SOP Training. At the start of each habitat assessment, the DMF QA/QC Analyst will train project partners on all aspects of field data collection and assist with a location literature review. Project partners will be accompanied on at least the first assessment trip by DMF project staff to continue the hands-on training and assist with the sample station selection.

Chain of Custody. Except for nutrient data analyzed at an external laboratory, all data will be recorded in the field on Form 4.1. The project manager for each assessment is responsible for maintaining a file for all field data sheets. Nutrient sampling will require a separate chain of custody form (Form 3.2).

Data Documentation. A separate Form 4.1 will be used for each sampling trip. The project manager will maintain a file for each assessment project and supervise the entry of the data into an annual Excel datafile for each location. Sampling stations should be labeled with a unique two or three letter/one number code (ex. SL-1) and the station position should be recorded with GPS.

Database Management. Data files will be saved on the DMF common server (W:\) and back-up files will be saved on primary server (P:\) of each field coordinator. The data classification will be updated by the QA/QC Analyst and care should be made to ensure the back-ups are consistent with the primary files. Once all possible review is completed and data has received a *Final* classification, the annual river datafile will be saved as read-only files in both the primary and common server.

Datafile Classification. The QA/QC Analyst should review the data and classify the QA/QC review status and data status using the classes listed below. The QA/QC status classes refer to the review stage for the entire datafile. The data status classes refer to the status of data following the QA/QC review.

QA/QC Status

1. Draft. Data processing is in progress, and QA/QC has not been conducted

2. Preliminary. Data processing is complete, but QA/QC is not complete. Data can be used for internal project summaries

3. Complete. All data processing and QA/QC review is completed

Data Status

1. Preliminary. Data have been entered or downloaded from field sheets, but the QA/QC review is not complete

2. Censored. Data are eliminated because of instrument failure or QA/QC performance

3. Conditional. Data are fully audited, and QA is complete, but have deficiencies that are documented and may limit use

4. Final. Data are fully audited, checked and acceptable

REPORTING OBJECTIVES FOR V-2

River herring habitat assessments at over 30 locations have been completed to date. This is a sufficient number of locations to begin analyses on data distributions and regional patterns. Habitat assessment reports under V-1 were consistent in reporting methods and formats. The following are recommendations made under QAPP V-2 for improvements to reporting objectives for individual assessment reports and project-wide data summaries.

1.) *Minimally Impacted Thresholds.* Data from ponds and lakes should be summarized by region and water body type to calculate percentile distributions (ex. 75th percentile) for each reference condition that will represent a threshold for "minimally impacted" habitat.

2.*) Impaired Thresholds.* Data from ponds and lakes should be summarized by region and water body type to calculate percentile distributions (ex. 25th percentile) for each reference condition that will represent a threshold for "Impaired" habitat.

3.) *Individual Assessment Reports.* Habitat assessment reports have been completed for 5 locations and published in DMF’s Technical Report Series. However, progress completing full reports has been slow and not supportive of the goal of quickly getting assessment data out to regulators and local users. Assessments at large waterbodies with regional significance can continue to use the TR Series formatting for reporting. Otherwise, reporting for most assessments is better served by brief memos that summarize *Completed* datafiles along with regional TR Series reports that combine assessments within major coastal drainage areas.

MAINTENANCE

Storage and Transportation. During the sampling season, instruments should be transported and stored in a carrying case. The case should be cushioned to prevent movement of the sonde during transport. The sensors should be protected in the calibration cup with a third volume of tap water. After each use, the sonde (with calibration cup attached) and display unit should be allowed to air dry on the bench top. After each marine deployment, all components should be cleaned with tap water. On a weekly basis, the carrying case should be dried out and the cable should be dried out and re-coiled. Cables should be carefully hand-coiled to loops no smaller than 1 ft diameter.

With-in Season. It should not typically be necessary to remove sensors from sonde during with-in season maintenance for freshwater deployments. A test-tube brush or toothbrush is suitable for dislodging sediment and organic deposits. The sensors can be soaked briefly in warm, soapy water prior to cleaning. With each cleaning between long-term deployments, inspect conductivity ports, DO anodes, and the glass bubble of pH sensor and refer to YSI operational manual for specific cleaning instructions. Wiper pads should be removed and cleaned (or replaced) following each long-term deployment. The DO membrane for sensor #6562 should be inspected at weekly calibrations for wear or damage and should be replaced routinely every 3-4 weeks.

Annual Maintenance. At the end of the sampling season, remove all probes and clean o-rings. Sensors should be cleaned and stored dry, except the DO probes should be stored in tap water, and the pH probe is stored in 2 M KCl. The pH probes can be stored for a month or less in tap water, but never in distilled water and should not be allowed to dry out. When sensors are re-installed for the start of the sampling season, replace o-rings as needed and lubricate all o-rings with a light application of silicon grease. Remove batteries from sonde during off-season storage.

TECHNICAL NOTES

Secchi Disk. Secchi disk depth is an easily measured parameter that has been used for decades around the world as a measure of water clarity and a relative indicator of water body health. The depth of Secchi disk measurements can provide information on light attenuation, suspended particles, and plankton production. When possible, Secchi disk depth should be recorded on the leeward and shady side of the boat or platform used by field staff. It is recognized that there will be occasions when it is not possible to record the measurement out of direct sunlight. These measurements can have increased visibility over a cloud cover or shaded condition. For these situations there is not much that can be done other than make a note on the conditions. The measurement recorded will be the average of the ascending and descending depths at which the disk cannot be seen. Because of varying eyesight among users, the same user should take all Secchi disk measurements on a given sample trip, and, if possible, for the entire season.

Dissolved Oxygen. The users of this QAPP should be aware that DO concentrations in water can vary dramatically throughout the day due to diurnal dynamics involving photosynthesis, respiration and temperature changes. Monthly grab samples taken at different times of the day can lead to biased average DO values for a water body. Continuous measurements are the only means to fully characterize DO trends throughout the warm months of the assessment period. It is recognized that continuous measurements will not be possible for most projects, and are limited to a single location and depth. Consideration should be given to deploying continuous water chemistry loggers in water bodies when project resources allow, and where grab samples identify DO concerns but result in marginal designations.

Water pH. Water pH is a measure of hydrogen ion concentration in water as an indicator of acidity. The negative log of hydrogen ion concentrations is reported as standard units (SU). Water pH at 7.0 is neutral, while values below 7.0 are acidic and values above 7.0 are basic. The pH of rainwater when at equilibrium with carbon dioxide is typically 5.65. Natural buffering in waterbodies tends to raise pH above the acidity contributed from rainfall. Aquatic plants take up carbon dioxide and hydrogen ions during photosynthesis. This process increases pH values in ponds as the day progresses with higher values near the surface. This condition is exacerbated in ponds with higher productivity. The diurnal dynamics of pH cycling creates a similar sampling dilemma and data interpretation challenge as dissolved oxygen. QAPP users should consider diurnal and depth interactions with water pH during reporting. The deployment of continuous water chemistry loggers can improve reporting of pH conditions in water bodies, although only for the given depth of deployment.

Global Positioning Systems Data. Projects should document the GPS units used for latitude and longitude measurements and confirm that units are recording decimal degrees units under datum NAD83 with suitable accuracy.

Environmental Data. Daily and monthly precipitation and monthly average air temperature should be recorded after the assessment period from a nearby weather station accepted by the NOAA's National Centers for Environmental Information (NCEI; formerly NOAA's National Climatic Data Center) http://www.ncdc.noaa.gov/oa/ncdc.html.
Monthly NCEI data during assessments should be compared to long-term station averages and departures from normal should be noted in the assessment report.

Several precipitation metrics can be used to relate to water chemistry data. To date, DMF assessments have used monthly and annual total precipitation, departures from normal, and a 3-day rainfall total (includes day of sample and two previous days).

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Form 4.1. River Herring Habitat Assessment Field Data Sheet, page 1 and page 2. The spacing of rows and columns can be customized for station information at each assessment location.

River Herring Habitat Assessment: Field Data Sheet (Form 4.1)

Location: **Organization:**

Date: **Field Crew:**

STATION

Name	Latitude	Longitude	Type	Max. Depth (m)	Notes

INSTRUMENT

Name	Model	Unit ID	Calibration (Date)	Notes

WATER CHEMISTRY

Station	Time	Depth (M)	Water Temp. (^oC)	Water Sp. Cond. (mS/cm)	Water pH	Water Turbidity (NTU)	Water D.O. (mg/l)	Water D.O. (% sat.)	Secchi Disk (m)

OUTLET

Name	Latitude	Longitude	Type	Width (m)	Outlet Depth- 25% (cm)	Outlet Depth- 50% (cm)	Outlet Depth- 75% (cm)	Channel Min. Depth (cm)	Staff Gauge (cm)

NOTES

FISH PASSAGE *(QAPP p. 68)*

Station No.

Water Body						
Outlet Type						
Downstream Impediment						
Upstream Impediment						
Impediment Type						
Designation *(BPJ)*						

Notes

STREAM FLOW *(QAPP p. 69)*

Discharge/Velocity						
Habitat Dewatering						
Supports Downstream Passage						
Supports Upstream Passage						
Impediment Type						
Downstream Reach Type						
Designation *(BPJ)*						

Notes

SPAWNING SUBSTRATE *(QAPP p. 60)*

Gravel (%)						
Sand (%)						
Silt (%)						
Periphyton (%)						
Vascular plant (%)						
Plant Abundance (rank 1-5)						
Invasive Plants (species)						

Notes

EUTROPHICATION *(QAPP p. 68)*

Plant Growth (water column, %)						
Plant Growth (substrate, %)						
Plant Growth (surface, %)						
DO (< 5.0 mg/L or ≥125%?)						
Water Clarity (<2.0 m SD?)						
TN/TP Sample (No.)						
Designation *(BPJ)*						

Notes

Section 5.0 Fish Kill Standard Operating Procedure

SCOPE AND APPLICATION

A fish kill is a sudden and significant death of large numbers of fish and shellfish, occurring in a defined area. Fish kills can occur at various times during the year and can result from natural causes or from anthropogenic activities. The location and natural resources affected by such events determine which agencies are responsible for initial response, investigation, and when necessary, sample procurement and testing as well as enforcement. This SOP will focus on diadromous fish resources that occur in marine and coastal river watersheds of Massachusetts but can also be applied to other marine fish and shellfish species that are jurisdictional to DMF.

Monitoring Objectives

The main purpose of Section 5.0 is to provide standardized protocols for documenting the occurrence of fish kills, and when possible, determining the causes and impacts of fish kills, and assessing the extent of damages to affected habitats and monetary value of diadromous resources. A supplementary goal of this SOP is to streamline the fish kill investigation protocols of DMF with those of *MassWildlife* (DFW), the Massachusetts Department of Environmental Protection Bureau of Waste Site Cleanup/ Emergency Response (MassDEP BWSC/ER) and the Massachusetts Department of Agricultural Resources (DAR) and other interested agencies, and to establish jurisdictional processes for notifications and first response, as well as the preparation, storage and chain-of-custody protocols for sample deliveries for laboratory testing. This SOP adopts direct guidance from the joint DFW and DEP Fish Kill SOP (MassDEP 2013) and the American Fishery Society fish kill guidance document (AFS 1992), and strives to relate our practices to MassDEP (2013).

Natural Fish Kills

Common causes of natural fish kills include oxygen depletion events during the winter and summer months, diseases and parasites, and thermal stress during the spawning season. Natural fish kills are most often the result of low dissolved oxygen (DO) concentrations created by a combination of environmental conditions and biological factors. Weather patterns, water temperature, plant growth, fish abundance and condition, along with the presence of viruses and bacteria, are all factors that can influence a DO-related fish kill, and at times be a primary driver in fish kills.

Many fish species can tolerate temporary reductions in DO, however, fish become stressed during prolonged periods of low DO and fish kills may result from insufficient respiration or susceptibility to viral or bacterial infections. A description of the symptoms and condition of species affected by oxygen depletions and other causes are described in Southwick and Loftus (2003 and 2017; Appendix 5A.1). Symptoms of oxygen depletion may include fish aggregating and gulping for air at the water surface or waterbody edges. Other symptoms of oxygen depletions may include a change in the clarity and color of the water and a foul odor may be released. Fish of all sizes are usually affected; however, smaller fish can survive longer under these conditions because they can have lower metabolic requirements and a greater gill surface to body ratio than larger fish. Various scenarios of DO-related fish kills are summarized below.

Nutrients and aquatic vegetation. Moderate levels of nutrients and aquatic vegetation are beneficial to fish populations. During the day plants utilize sunlight to produce oxygen via photosynthesis. During the night plants and animals consume oxygen and produce carbon dioxide (CO_2). In waterbodies with excessive plant growth, the amount of oxygen produced during the day may not be sufficient to sustain aquatic life when multiple nights are followed by overcast days. Fish populations become stressed when oxygen levels are depleted and fish kills may result from these conditions which commonly occur during warm months (June through September), known as “summer kills.”

Winter fish kills can occur in waterbodies with excessive plant growth. Ice and snow cover for extended periods of time cause anoxic (oxygen depleted) conditions along the bottom. In the absence of sunlight penetration through the ice, plants stop producing oxygen and oxygen levels become depleted due to the subsequent decay of dead plants and other organic material. Winter fish kills may not be discovered until after the ice has melted.

Planktonic algae. Phytoplankton can become overabundant in waterbodies that receive excessive nutrients from substrate sediments, surface water inputs, groundwater, or runoff. High phytoplankton density in shallow waters may create the same oxygen cycling process generated by rooted plants. If the phytoplankton die-off, the loss of DO generation and rapid decomposition can cause oxygen depletion and a fish kill may result.

Turnover. In temperate regions, turnover is a natural process that occurs in ponds and lakes. During summer, the heat and calm weather causes the water to stratify into layers. The upper layer (epilimnion) is exposed to the sun and receives oxygen from the atmosphere and photosynthesizing plants. The metalimnion is a thin layer where temperature and density changes rapidly. The hypolimnion is a cold, denser, lower layer that lacks sunlight and resists mixing with the upper layers. In this layer, bacteria utilize oxygen to decompose dead animals and plants which can lead to anoxia due to lack of mixing with the upper layers. Turnover occurs naturally in the autumn as the epilimnion cools and the layers gradually begin to mix creating a uniform temperature (commonly near 10°C) throughout the waterbody. In winter, inverse stratification occurs as surface waters cool below the temperature of water near the bottom.

In spring, a combination of increasing wind action after ice-out and rising warmer water at the bottom increases the size of the epilimnion and decreases the size of the hypolimnion. Under certain environmental conditions, turnover can result outside of the natural changing of seasons. For example, the high winds of thunderstorms can cause sudden mixing of anoxic water from the hypolimnion with the epilimnion. The result is a rapid reduction in DO at water column depths occupied by fish which may induce a fish kill.

Thermal Stress. Fish kills caused by thermal stress are common in the spring. Prolonged inactivity during winter months, followed by the stress of spring spawning, leave adults in a weakened state and less resilient to environmental changes. Sudden periods of hot weather can rapidly increase water temperatures in shallow areas of where spawning may occur. The compounding stress may lead to lethal conditions as the adults may become susceptible to infections from bacteria and parasites.

Surface Flow Reduction. Fish kills caused by low flows can occur during the summer months when drought conditions often result but can also occur during abnormally dry periods in the spring and autumn. Migratory species, such as river herring and American shad are susceptible as they migrate between fresh and marine waters. Sudden changes in flow levels (such as flash-flooding immediately followed by drought conditions) can induce fish kills as fish can become stranded in shallow water or dried-up sections of rivers or susceptible to low DO.

<u>Anthropogenic fish kills</u>

Fish kills can also result from direct responses to human influences on water or habitat quality or indirect or delayed influences from pollutants. In contrast to various natural causes that often involve single species and a certain size range, fish kills resulting from anthropogenic activities often affect multiple species of all sizes.

Water usage and withdrawals. Numerous waterbodies in the Commonwealth are utilized as public water supplies by municipalities or as irrigation reservoirs for agricultural. The demand for water increases typically during summer but can continue into the fall during droughts. Various scenarios of water usage and withdrawals during these periods can cause fish kills of juvenile diadromous fish. Water withdrawals can impact young-of-year (YOY) river herring as water quality and suitable nursery habitat are reduced. In addition, water withdrawals and diversions for agriculture irrigation can cause physical damage to fish during pumping and strand emigrating YOY in shallow channels and dewatered areas.

Pollution. In pollution-related events, the magnitude of the fish kill is variable and depends on the concentration of pollutants and tolerance of species to different pollutants as some species are more tolerant than others. Pollutants can enter waterbodies either through direct ("point-source") input or indirectly ("non-point source").

Point Source. The term "point source" as defined in section 502(14) of the Clean Water Act means any discernible, confined and discrete conveyance, including but not limited to any pipe, ditch, channel, tunnel, conduit, well, discrete fissure, container, rolling stock, concentrated animal feeding operation, or vessel from which pollutants are or may be discharged (USEPA 2014). Various examples of point source pollution include discharge of industrial wastes from factories, chemicals from industrial plants, sewage outflow pipes, and concentrated livestock operations.

Aquatic Herbicides. Herbicide application is a point source pollutant commonly used to treat excessive aquatic plant growth. In Massachusetts, a variety of herbicide agents including Flouridone (Sonar), Glycosphate (Rodeo) have been applied to waterbodies for the purpose of controlling various species of invasive flora such as *Hydrilla sp.*, milfoil and fanwort. Results of various case studies of herbicide treatments in Massachusetts waters are referenced in Mattson et al. (2003). Approval to apply chemicals to control nuisance aquatic vegetation is granted under authority of the Mass*DEP* General Laws c. 111, s. 5E (Mass*DEP* 2016). Treatment should be administered gradually and at certain times of the year. Treatments of dense invasive plants can create excess decaying plant matter. This in turn triggers rapid growth of bacteria and can create lethal conditions for a variety of aquatic life as oxygen levels in the water become depleted.

Piscicides. Use of piscicides is a tool for fisheries managers to manipulate fish communities for a variety of purposes including reclamation (Schnick 1974), controlling or eradicating harmful exotic fish, quantification of populations (Parker 1970; Shireman et al. 1981), disease control, or to restore endangered species (Bettoli and Maceina 1996). Application of piscicides such as rotenone is effective in the complete eradication of undesirable fish communities or for sampling a fish population (Finlayson et al. 2000). Few piscide applications have occurred in Massachusetts in recent decades.

Nonpoint Source. Nonpoint source pollution is the transfer of pollutants into waterbodies from many diffuse sources including runoff from land, precipitation, atmospheric deposition and drainage (USEPA 2014). Nonpoint sources of pollution are common in developed areas and can include the following:

-Excess fertilizers, herbicides and pesticides from agricultural lands, golf courses and residential areas

-Toxic chemicals from urban runoff and energy production

-Sediment from construction sites, agriculture and forest lands, and eroding streambanks

-Bacteria and nutrient input from septic systems and livestock operations

-Hydromodification (alteration of the natural flow of water through dams, landscaping, stream channel modification, streambank and shoreline erosion control measures

Water intake operations. In many areas, ponds, lakes, rivers and estuaries serve as water supplies for agricultural operations, water treatment, desalination and power generation. Such operations rely on intake systems to withdraw water for irrigation and in the case of power plants for cooling system operations. Conventional water intake systems are fitted with screens to prevent large organisms and debris from entering and obstructing the systems, however, they can impact various aquatic organisms and habitat. Impacts of water intakes on diadromous fish populations have been well documented (Christensen et al. 1977; Goodyear 1977; McCaughran 1977; Saila and Lorda 1977; Swartzman et al. 1977; Hanson et al. 1977) Adverse effects of water intake systems on aquatic organisms can be divided into the following major categories: a) *entrainment* of fish eggs and larvae and other small organisms; b) *impingement* of larger organisms on the intake screening systems; and c) impacts from discharge of thermal effluent on the aquatic community and habitat.

Impingement and entrainment. Impingement occurs when organisms sufficiently large to avoid passing through the screens are trapped against them by the force of the flowing water. Juvenile and adult fish may be killed quickly due to mechanical abrasion and suffocation or may become stressed and eventually killed due to exposure to disease and predation. Entrainment occurs when marine organisms small enough to pass through the screens enter or are drawn into the intake system. Entrainment often occurs to juvenile species and mortality is high as they pass through to the treatment facilities, turbines or irrigated fields with various mortality rates reported for adults (Chittenden 1973).

Thermal effluent discharge. Many power plants require water for their cooling systems however, this results in heated water being released back into the receiving environment. Sedentary species (i.e. shellfish), juvenile fish (Ruelle et al. 1977, Beitinger et al. 1999; Madden et al. 2013) and habitats in close proximity to the discharge site can be impacted due to exposure to processed water that has higher than ambient temperature, ash from fossil fuel plants, or radioactive wastes from nuclear plants.

Water Drawdowns. The drawing down of water levels at the control structures of lakes, ponds, and reservoirs is a management practice that can be used for specific goals such as invasive plant control or water quality improvements. The timing is typically the winter to avoid impacts to aquatic life. Fish kills are not common during drawdowns with standard practices yet can occur outside of winter months, from isolated dewatering, and disruption of hypolimnetic anoxic waters.

Jurisdictional Boundaries

This section directs DMF staff in responding to reports of diadromous fish kills, and can serve as a guide for all fish, shellfish and other invertebrates found within Massachusetts marine and estuarine waters as well as “coastal waters” (rivers and impoundments) as described in the Massachusetts General Laws (MGL) Chapter 130, Section 1:

Chapter 130: § 1. Definitions; rules of construction. “Coastal waters”, all waters of the Commonwealth within the rise and fall of the tide and the marine limits of the jurisdiction of the Commonwealth, but not waters within or above any fishway or dam nor waters above any jurisdictional boundary legally established pursuant to MGL Chapter 130: § 5 in rivers and streams flowing into the sea.

The provisions of MGL Chapter 130, § 5 empower the Directors of DMF and DFW to determine the jurisdictional boundaries in rivers and streams flowing into the sea for purposes of fisheries management authority. Approximate locations delineating the jurisdictional boundaries between the two agencies are listed in Appendix 5A.2. In most coastal systems of the Commonwealth, the boundary separating the jurisdiction between the two agencies is located either at the “head of tide” or at the first obstruction on a coastal stream. Exceptions to these boundaries are described in a Memorandum of Understanding (MOU) between DFW and DMF prepared in 2003 for the purposes of creating a uniform regulatory process for the two agencies. The provisions of the MOU recognize that management of anadromous striped bass (*Morone saxatilis*) and river herring, alewife (*Alosa pseudoharengus*) and blueback herring (*A. aestivalis*) shall fall within the regulatory authority of MA DMF in all waters of the Commonwealth. Jurisdiction for all other diadromous species is split between DFW and DMF at the coastal boundaries.

<u>Reporting a Fish Kill</u>

First Response. For fish kills involving marine resources under DMF jurisdiction (as described in MGL 130 s. 1), staff biologists under the Recreational and Diadromous Fisheries Program are designated as the primary responders. In certain cases, investigations can involve other state agencies, municipalities and federal agencies and may require the expertise of professionals from several disciplines. For example, the U.S. Endangered Species Act gives the U.S. Fish and Wildlife (USFWS) Service and the National Marine Fisheries Service (NMFS) the authority to investigate fish kills if endangered or threatened fish are harmed or if the affected area is critical habitat for such species (DOC-NOAA 1999). In the event of fish kills that involve known pollutants, DEP should be notified and monitoring on the extent and value of marine and diadromous resource damage should be coordinated with DEP.

The following steps to take following the notification of a fish kill were adopted from the joint MassDEP and FWE fish kill SOP (MassDEP 2013) with the addition of references to specific DMF actions:

<u>Notifications of fish kills from the public</u>

1. All reports of fish kills in coastal waters received by DMF staff should be reported Diadromous Fish Project staff in Gloucester (for the region of Newbury to Hull) and New Bedford (for the region of Cohasset to RI Border).

2. The Diadromous Fish Project staff will determine if the resources impacts are diadromous fish or other jurisdictional marine species. If the resources are not diadromous fish, then regional staff from the Recreational Fish Project or Shellfish Project will be contacted. Once the fish kill responsibility is assigned, DMF staff should contact the DFW Fish Kill Coordinator.

3. Call the DFW Fish Kill Coordinator at (508) 389-6334 (office) or at the DFW Fish Kill Notification phone at (508) 450-5869. If the DFW Fish Kill Coordinator is not available, leave a message including the name and number of the witness and the location of the fish kill. .

4. The lead DMF staff should document the event by opening a Fish Kill Notification form (available on DMF Wiki site and W:\drive).

5. If the fish kill may involve pollutants or other anthropogenic influences, the lead DMF staff should contact the DEP Emergency Response team as described in the following section.

6. For fish kills that require an investigation (based on size/severity/species) the MA Environmental Police (MEP) should be notified. Contact the MEP Hingham Radio Room at 1-800-632-8075.

7. Communications with media outlets must be first approved by the Department of Fish and Game press secretary.

<u>Notifications of pollution-related fish kills</u>

1. Upon investigation of any fish kill in which pollution is suspected, the investigating agency will contact DEP via the DEP 24-hour Emergency Response number (888) 304-1133. All fish kill notifications and referrals received by the DEP BWSC ER, including marine and estuary fish kills, will be considered a Significant Incident Category 9 under the BWSC Significant Incident SOP. BWSC ER personnel will take the lead on investigating the source, sample collection and delivery, and begin case development.

2. In the case of pollution-related fish kills that appear to be the result of negligent or intentional activity, BWSC will contact DEP Environmental Strike Force (ESF). ESF personnel will take the lead on investigating the source, sample collection and delivery and begin case development.

3. For fish kills resulting from pesticide applications, the Massachusetts Department of Agricultural Resources (DAR) is the lead agency responsible for the administration of the Pesticide Control Act (M.G.L. Chapter 132B, Section 1-15), including the administration of the Federal Insecticide, Fungicide and Rodenticide Act. DMF will notify the fish kill response coordinator for the DAR (Pesticide Bureau) in cases where pesticides are likely causal factors. DAR personnel will be responsible for the collection and analysis of water and fish samples, and identifying contaminants, sources, and responsible parties.

<u>Response and Assessment Procedures</u>

Upon arrival at the site, and confirmation that a fish kill has occurred or is occurring, the following procedure should be followed.

1. Talk to any witnesses or observers. Take a statement from any person at the scene who may be have pertinent information. Record name and contact phone numbers.

2. Consult with local officials such as the local Herring Warden or Shellfish Constable, Department of Natural Resources (DNR) official, Municipal Board of Health (BOH), or Conservation Commission as necessary.

3. Determine and delineate the extent of the kill area on a chart or map. As needed, stakes, marker tape or buoys can be deployed to temporally mark the extent of the kill area(s).

4. Inspect the site to determine the cause of the kill. If visual observations or other evidence confirm an obvious chemical contaminant contact DEP ER who will take the lead on the investigation. Do not sample the area until ER personnel are onsite and give authorization.

5. If no chemical contamination is implicated or if clearance to sample is given by DEP personnel, proceed with an investigation. Begin filling out the Fish Kill Investigation Report Form (Appendix 5A.3) and continue to use it throughout site inspection.

6. Photographs should be taken of the site landscape and dead or affected fish and any other materials suspected of being associated with the fish kill. The date, time, and location of sequential photographs and the name of the photographer should be recorded in a field notebook.

7. Water quality measurements should be made along the entire extent of the kill and in nearby un-impacted areas (reference area). Take a minimum of three measurements in each area. Measure water quality at a center point of the fish kill area and at 25 m to either side of the center point. Add measurements at 25 m intervals as needed. Measure 5 m from the shoreline at 0.3 m depth and 1.0 m depth. Document any changes in the measurement locations necessitated by site-specific conditions. Document the exact location of each measurement in the field notebook and the Fish Kill Investigation Report Form. Water quality instruments should follow QA/QC procedures provided in SOP 2.0. Testing should include the following parameters:

a. Water temperature
b. DO
c. Salinity
d. Specific conductivity
e. pH
f. Turbidity

8. *Biosample collection.* In cases of fish kills in which pollution is not suspected, the following protocols should be followed in the collection and preparation of biological samples:

a. Wear gloves and other protective wear when collecting samples.

b. Place live samples in ambient water (do not use water from another source).

c. If fish are dead, place 5-10 of each species (in freshest condition) in separate Ziplock bags and cover with ice to be subsequently frozen. Make sure each bag is labeled with a Chain of Evidence (COE) tag.

d. Inspect and dissect a few of the fish samples saved. Record internal and external observations of physical abnormalities (refer to the Fish Kill Investigation Form 5A.3 for a list of physical conditions).

e. Record all relevant observations in the field notebook and on data sheets.

f. Photographs should be taken of dead or affected fish and other organisms.

g. Specimens should be identified by species, and size and weight measurements should be made and recorded on the Fish Kill Subsampling Form (Appendix 5A.4). Where possible, life stage, sex and maturity information should be recorded.

9. *Fish Number Estimation.* The investigator should attempt to derive an estimate of the total number of organisms affected as the result of the fish kill. The appropriate method of estimation is dependent on the size, location, and accessibility of the fish kill area. In many cases it will not be possible to obtain a complete enumeration due to large numbers of fish dispersed a wide area (Ryon et al. 2000). Therefore, various survey sampling methods described by Labay and Buzan (1999) and Southwick and Loftus (2017) can be performed.

a. <u>Complete enumeration</u>: Complete counts can be applicable for small-scale fish kills that occur within a defined area that is accessible to investigators.

b. <u>Survey Sampling:</u> Survey sampling is applicable for large-scale fish kills in which complete counts are is not possible. Survey sampling relies on conducting counts and collecting samples from a representative portion of the area affected to derive an estimate of the entire kill. For statistical and legal purposes, the sampling method must be as defensible as possible. The following guidelines should be applied for two common methods of survey sampling: shoreline counts and area sampling.

-Sample units are areas in which all fish are counted and measured and expanded over the entire area affected to derive a total estimate of fish killed.

-Sample units must be chosen at random to avoid introducing bias in the sampling design and estimates of the total number of fish killed. Select a suitable random number generator and document the usage.

-Precision depends on sample size and number of fish counted and is reflected by the coefficient of variance (CV) in the estimate. Sample designs should seek a CV of ≤25% and document the factors the cause exceedances.

<u>Shoreline count</u>: In coastal areas which are affected by both tide and wind, dead fish usually accumulate along the shoreline. Determine the length of the affected area. Subdivide the shoreline by appropriate (to scale) sampling segments of fixed length. Determine the total number of segments. Randomly select at least five segments to sample. All dead fish from selected segments are counted and measured, and the counts are expanded with a subsampling ratio (*n*) over the entire affected shoreline to estimate the total number of fish killed.

Apply the following equation to generate the subsampling ratio:

$n = N_t/N_s$

where N_t is the total number of segments and N_s is the segment numbers to be sampled.

<u>Area sampling</u>: May be applicable for fish kills that occur in shallow ponds and inlets as well as open water such as embayments where counts must be made by boat. Set at least three transects that span the affected area. Determine the length of each transect. Randomly select along each

transect at least five segments (quadrats) of standard dimensions in the range of 1-5 m^2. Therefore, the minimum number of quadrats sampled would be 15.

All dead fish from selected quadrats are counted and measured, and the counts are expanded with a subsampling ratio (*n*) over the entire affected area to estimate the total number of fish killed. Apply the following equation to generate the subsampling ratio:

$$n = N_q/N_s$$

where N_q is the total number of quadrats and N_s is the transect numbers to be sampled. All dead fish from selected quadrats are counted and measured, and the counts are expanded over the entire affected area to estimate the total number of fish killed.

Sample Variance. The sample variance should be calculated from the fish counts recorded for N_s and used to derive CV. In cases, where large numbers of segments with zero fish are counted, variance can be calculated from the geometric mean of N_s.

Fish Kill Valuations

The assessments for large-scale fish kills of fish, shellfish and invertebrate species under DMF management jurisdiction should include monetary valuations. Refer to Southwick and Loftus (2017), a guide produced by the American Fishery Society, for valuations on fish and other aquatic life. Replacement and restoration costs include the acquisition and transportation of fish from hatcheries or donor system to recipient waters, as well as the associated costs of vehicles, fuel, water, personnel and equipment. In addition, the costs of conducting the investigation including personnel, transportation, field equipment, sampling supplies and disposal of dead fish should be documented. Such information should be documented electronically (see Reporting) and be made available upon request for enforcement and litigation cases.

Safety Equipment and Sampling Supplies

All DMF staff that responds to fish kill are required to possess and employ safety and sampling equipment. In addition, all responders must have the required field data sheets for data and sample recording, photo, site and pollution documentation. Water chemistry instruments should be field calibrated before use following the specifications of SOP 2.0 in this QAPP. DMF staff should also have up-to-date contact information of town herring wardens and Department of Natural Resources staff, ELE and MassDEP personnel.

Reporting

All DMF fish kill investigations will be documented in a Fish Kill Investigation Report Form and Fish Counting Record Form (5A.3 and A5.4). Essential information will include initial time of notification, response actions, notes on observations, sampling times, names and contact information of witnesses, and other agency staff. These forms document the investigative process and may undergo judicial review if a polluter is brought to court for damages. In addition to paper filing, all fish kills will be documented in an Excel fish kill datafile with information fields related to the above-mentioned forms.

Draft reports should be completed and submitted within 48 hours or the next two business days following the fish kill and an electronic copy sent the Diadromous Fish Project Leader depending on the severity of the fish kill, and upon request, these reports will be made available to DFW, MassDEP, ELE, and EOEEA for documentation, enforcement and litigation. In latter cases (pollution-related or intentional activities) where enforcement and litigation against and individual or party is warranted, a detailed final report is required. In such cases, DMF fish kill investigators are required to contribute the following documents:

a. Copy of the original field fish kill report form

b. Summarized discussion of the investigative procedure, findings and conclusions

c. Maps delineating kill area and sampling sites

d. Copies of Chain of Evidence Record

e. Laboratory analyses results

f. Pertinent biological data collected such as tables of fish species collected and their respective sizes

g. Literature references relative to known toxicities of the causative agent

h. Reference to state and federal laws violated

i. Monetary values of fish and other organisms killed and the cost of investigation (Southwick and Loftus 2017)

j. Photographs documenting the kill

k. Recommended action(s)

<u>Follow-up Investigations and Reporting</u>

Follow-up to fish kill investigations will be handled on a case by case basis. Information, data, analyses and recommendations from subsequent investigations will be recorded and archived (both paper and electronic files) with the original case at either the DMF New Bedford or Gloucester facilities and shall be made available upon request by agencies for enforcement and litigation cases.

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Appendix 5A.1. Fish kill site conditions and possible causes (source: Southwick and Loftus 2003).

Condition(s)	Possible cause
Fish gulping for air at the surface Low dissolved oxygen Green water	Oxygen depletion due to organic matter input from sewage treatment plants, livestock feedlot, irrigation run-off, or algal bloom (green water)
Fish gulping for air at the surface Adequate dissolved oxygen Discolored water	Restored oxygen levels after depletion by organic matter from sources (above) Toxic concentrations of Ammonia Toxic algal bloom (discolored water)
Fish dying after heavy rain	Run-off of pesticides or other chemicals from adjacent agricultural fields or discharged from spraying
Oily sheen on water	Seepage from refinery, drilling operation or pipeline; or petroleum spill from vessel or truck
Stream bank and substrate covered with an orange substance; high water conductivity	Discharge of brine water from drilling operations
Water has low pH (with or without orange discoloration of substrate); high water clarity	Discharge of acidic water from coal mine or chemical spill. Coniferous tree plantations close to water
Small fish dead along shoreline Sub-freezing air temperature	Excessive cold
Small fish dead below a dam or industrial plant discharging heated water	Fish killed by entrainment through intake valves, turbines or from exposure to thermal shock
Kill restricted to one species or size class	Spawning stress, disease pathogens

Appendix 5A.2. Approximate locations of boundaries (head of tide; first obstructions) for jurisdictional purposes between DMF and DFW for Massachusetts coastal streams (per MGL Chapter 130, Section 1). Information on the presence of diadromous species at these locations is available in Evans et al. (2011) and Reback et al. (2004a,b; 2005a,b).

Region	Watershed	Stream	Boundary	City/Town	River Mile	GPS Latitude	GPS Longitude
North Coastal	Merrimack	Merrimack River	Interstate 495	Haverhill	16.1	42° 46' 06.044" N	71° 07' 12.089" W
North Coastal	Merrimack	Powwow River	Mill Street Dam	Amesbury	7.1	42° 51' 24.608" N	70° 55' 46.359" W
North Coastal	Merrimack	Back River	Clarks Pond Dam	Amesbury	0.4	42° 51' 37.663" N	70° 55' 35.866" W
North Coastal	Merrimack	Artichoke River	Emery Lane (Curzon's Mill) Dam	Newburyport	1.2	42° 49' 09.330" N	70° 56' 14.603" W
North Coastal	Merrimack	Indian River	Mill Pond Dam	West Newbury	2.9	42° 48' 23.727" N	70° 58' 01.553" W
North Coastal	Merrimack	Shawsheen River	Rte. 133 Dam	Andover	25.0	42° 40' 20.522" N	70° 08' 58.243" W
North Coastal	Merrimack	Spickett River	Spickett River Dam	Lawrence	12.7	42° 42' 26.609" N	70° 08' 52.669" W
North Coastal	Parker	Little River	Hanover Street	Newbury	2.2	42° 47' 24.097" N	70° 52' 35.068" W
North Coastal	Parker	Parker River	Woolen Mill Dam/Central St.	Newbury	9.3	42° 45' 00.072" N	70° 55' 44.862" W
North Coastal	Parker	Mill River	Jewel Mill Dam	Rowley	4.2	42° 44' 20.829" N	70° 54' 01.723" W
North Coastal	Parker	Egypt River	Munic. Elect. Generating Plant	Ipswich	5.3	42° 41' 53.259" N	70° 52' 09.286" W
North Coastal	Ipswich	Ipswich River	Ipswich Mills Dam	Ipswich	3.7	42° 40' 39.110" N	70° 50' 15.572" W
North Coastal	North Coastal	Essex River	Elevation change (Apple Street)	Essex	1.0	42° 37' 30.514" N	70° 47' 24.096" W
North Coastal	North Coastal	Ebben Creek (Essex R.)	Grove Street	Essex	1.1	42° 37' 29.011" N	70° 45' 50.075" W
North Coastal	North Coastal	Walker Creek	Route 133	Gloucester	2.0	42° 37' 27.034" N	70° 44' 15.048" W
North Coastal	North Coastal	Alewife Brook	Cherry Street	Gloucester	1.2	42° 37' 48.089" N	70° 40' 12.092" W
North Coastal	North Coastal	Goose Cove	Falls above Denniston Street	Gloucester	0.0	42° 39' 07.120" N	70° 39' 54.417" W
North Coastal	North Coastal	Langsford Pond	Route 127 Spillway	Gloucester	0.1	42° 39' 39.842" N	70° 40' 15.568" W
North Coastal	North Coastal	Mill Brook	King Street Dam	Rockport	0.1	42° 39' 30.550" N	70° 37' 23.693" W
North Coastal	North Coastal	Sawmill Brook	Frank Street Culvert	Rockport	0.5	42° 38' 16.246" N	70° 36' 36.842" W
North Coastal	North Coastal	Sleepy Hollow Pond	Atlantic Street Culvert	Gloucester	0.6	42° 38' 48.394" N	70° 41' 53.121" W
North Coastal	North Coastal	Little River	W. Gloucester Water Treat. Facility	Gloucester	1.4	42° 36' 38.252" N	70° 42' 29.395" W
North Coastal	North Coastal	Fernwood Lake	R.R. Tracks (Lower Banjo Pond)	Gloucester	0.1	42° 37' 00.300" N	70° 41' 28.860" W
North Coastal	North Coastal	Buswell Pond	Duck Pond Culvert	Gloucester	0.1	42° 35' 49.015" N	70° 41' 08.085" W
North Coastal	North Coastal	West Pond	Shore Road Culvert	Gloucester	0.0	42° 34' 30.581" N	70° 42' 30.557" W
North Coastal	North Coastal	Chubb Creek	None	Beverly/Manchester	1.0	N/A	N/A
North Coastal	North Coastal	Bass River	Dam above Elliot Street	Beverly	1.6	42° 33' 30.838" N	70° 53' 17.332" W
North Coastal	North Coastal	Porter River	None	Danvers	1.7	N/A	N/A
North Coastal	North Coastal	Crane River	Mill Pond Dam	Danvers	1.8	42° 33' 33.396" N	70° 56' 32.043" W
North Coastal	North Coastal	Crane Brook	Interstate 95	Danvers	1.6	42° 33' 34.003" N	70° 58' 25.069" W
North Coastal	North Coastal	Porter Brook	Poplar Street	Danvers	0.0	42° 34' 05.037" N	70° 55' 40.031" W
North Coastal	North Coastal	Forest River	Railroad Bridge	Salem/Peabody	1.1	42° 29' 32.072" N	70° 54' 29.071" W

Region	Watershed	Stream	Boundary	City/Town	River Mile	GPS Latitude	GPS Longitude
North Coastal	North Coastal	Proctor Brook	None	Salem/Peabody	5.6	N/A	N/A
North Coastal	North Coastal	North River	Howley Street	Salem	1.5	42° 31' 27.082" N	70° 55' 07.035" W
North Coastal	North Coastal	Shute Brook	None	Saugus	2.0	N/A	N/A
North Coastal	North Coastal	Saugus River	Hamilton Street	Saugus	2.5	42° 27' 56.015" N	71° 00' 15.044" W
Boston Harbor	Mystic	Mystic River	Amelia Earhart Dam & Locks	Somersett/Everett	1.7	42° 23' 41.782" N	71° 04' 32.072" W
Boston Harbor	Charles	Charles River	Charles River Locks	Boston	0.9	42° 22' 06.924" N	71° 03' 42.812" W
Boston Harbor	Neponset	Neponset River	Baker Chocolate Factory Dam	Milton	4.2	42° 16' 14.096" N	71° 04' 07.531" W
Boston Harbor	Weymouth/Weir	Furnace Brook	None	Quincy	1.9	N/A	N/A
Boston Harbor	Weymouth/Weir	Town River	Monroe Field Culvert	Quincy	2.0	42° 15' 02.521" N	70° 59' 33.070" W
Boston Harbor	Weymouth/Weir	Smelt Brook	R.R. Culvert	Weymouth	0.13	42° 13' 17.282" N	70° 50' 04.374" W
Boston Harbor	Weymouth/Weir	Fore/Monatiquot River	McCusker Road Culvert	Braintree	5.5	42° 13' 16.016" N	70° 58' 58.974" W
Boston Harbor	Weymouth/Weir	Back River	Railroad Bridge	Weymouth	2.5	42° 13' 09.092" N	70° 55' 23.024" W
Boston Harbor	Weymouth/Weir	Broad Cove	None	Hingham	0.5	N/A	N/A
Boston Harbor	Weymouth/Weir	Weir River	Foundry Pond Dam	Hingham	2.7	42° 15' 48.794" N	70° 51' 38.082" W
Boston Harbor	Weymouth/Weir	Straits Pond	Straits Pond Tidegate	Hull/Cohasset	1.0	42° 15' 37.146" N	70° 50' 40.373" W
South Coastal	South Coastal	Little Harbor	Little Harbor Tide Gate	Cohasset	0.9	42° 15' 15.207" N	70° 48' 37.263" W
South Coastal	South Coastal	Musquashcut Brook	Musquashcut Pond Tide Gate	Scituate	1.2	42° 13' 31.113" N	70° 45' 34.019" W
South Coastal	South Coastal	Bound Brook	Hunters Pond Dam	Scituate/Cohasset	0.0	42° 13' 22.798" N	70° 47' 19.747" W
South Coastal	South Coastal	First Herring Brook	Old Oaken Bucket Pond Dam	Scituate	0.0	42° 10' 39.404" N	70° 45' 00.941" W
South Coastal	South Coastal	North River	Route 3 Northbound	Pembroke	6.5	42° 06' 58.022" N	70° 46' 40.089" W
South Coastal	South Coastal	Second Herring Brook	Gordon Pond Dam	Norwell	0.3	42° 09' 04.692" N	70° 47' 16.927" W
South Coastal	South Coastal	Third Herring Brook	Tiffany (Tack Factory) Pond	Norwell/Hanover	1.1	42° 07' 21.669" N	70° 48' 32.744" W
South Coastal	South Coastal	Indianhead River	Elm Street Dam	Hanover/Pembroke	2.0	42° 06' 01.128" N	70° 49' 26.429" W
South Coastal	South Coastal	Herring Brook	Barker Street Dam	Pembroke	2.2	42° 04' 32.359" N	70° 48' 02.697" W
South Coastal	South Coastal	Robinson's Creek	Howland Pond Dam	Pembroke	0.1	42° 05' 56.874" N	70° 47' 34.827" W
South Coastal	South Coastal	Macombers Creek	Damon's Point Road Culvert	Marshfield	1.4	42° 09' 05.309" N	70° 43' 42.556" W
South Coastal	South Coastal	South River	Willow Street	Marshfield	6.0	42° 05' 35.014" N	70° 42' 43.097" W
South Coastal	South Coastal	Green Harbor River	Green Harbor River Tide Gates	Marshfield	0.6	42° 05' 10.980" N	70° 39' 02.536" W
South Coastal	South Coastal	West Brook	North Hill Marsh Bog Sluice	Duxbury	1.0	42° 02' 48.533" N	70° 42' 22.934" W
South Coastal	South Coastal	Bluefish River	Amory Dam	Duxbury	1.6	42° 02' 17.568" N	70° 40' 32.983" W
South Coastal	South Coastal	Island Creek	Mill Pond Fishway	Duxbury	0.8	42° 01' 00.633" N	70° 42' 38.443" W
South Coastal	South Coastal	Halls Brook	Mill Pond Dam	Kingston	0.3	41° 59' 59.697" N	70° 43' 34.971" W
South Coastal	South Coastal	Jones River	Main Street	Kingston	1.3	41° 59' 45.078" N	70° 43' 23.011" W
South Coastal	South Coastal	Laundry Brook	Brook Street Culvert	Kingston	0.1	41° 59' 18.437" N	70° 43' 45.739" W
South Coastal	South Coastal	Smelt Brook	Foundry Pond Dam	Kingston	0.4	41° 59' 09.301" N	70° 42' 35.618" W
South Coastal	South Coastal	Town Brook	Water Street Dam	Plymouth	0.0	41° 57' 21.978" N	70° 39' 42.942" W
South Coastal	South Coastal	Shingle Brook	Howland Pond Dam	Plymouth	0.3	41° 55' 33.774" N	70° 36' 48.901" W
South Coastal	South Coastal	Eel River	Hayden Mill Pond Control	Plymouth	2.2	41° 55' 26.832" N	70° 37' 17.105" W
South Coastal	South Coastal	Beaver Dam Brook	Bog Reservoir Sluice	Plymouth	1.8	41° 54' 58.869" N	70° 34' 11.974" W
South Coastal	South Coastal	Indian Brook	Indian Brook Pond Dam	Plymouth	0.5	41° 53' 13.509" N	70° 32' 14.261" W

Region	Watershed	Stream	Boundary	City/Town	River Mile	GPS Latitude	GPS Longitude
South Coastal	South Coastal	Savery Pond	Salt Pond Control Structure	Plymouth	0.5	41° 50' 38.526" N	70° 32' 26.734" W
South Coastal	South Coastal	Monument River	Canal Culvert	Bourne	0.0	41° 16' 17.684" N	70° 33' 47.899" W
Cape Cod Bay	Cape Cod	Mill Creek	Sandwich Grist Mill Dam	Sandwich	2.1	41° 45' 27.531" N	70° 30' 01.498" W
Cape Cod Bay	Cape Cod	Maraspin Creek	Commerce Road	Barnstable	0.7	41° 42' 13.078" N	70° 17' 17.072" W
Cape Cod Bay	Cape Cod	Boat Cove Creek	Mill Pond Dam	Barnstable	2.2	41° 42' 33.151" N	70° 22' 54.822" W
Cape Cod Bay	Cape Cod	Whites Brook	Matthews Pond Outlet	Yarmouth	1.2	41° 42' 45.316" N	70° 13' 27.741" W
Cape Cod Bay	Cape Cod	Sesuit Creek	Route 6A	Dennis	1.5	41° 44' 41.083" N	70° 10' 24.072" W
Cape Cod Bay	Cape Cod	Quivett Creek	Route 6A	Dennis	1.5	41° 44' 41.040" N	70° 08' 42.064" W
Cape Cod Bay	Cape Cod	Stoney Brook	Lower Mill Pond Dam	Brewster	1.8	41° 44' 40.473" N	70° 06' 45.011" W
Cape Cod Bay	Cape Cod	Rock Harbor Creek	Rock Harbor Road Culvert	Orleans	1.4	41° 47' 50.100" N	69° 59' 29.700" W
Cape Cod Bay	Cape Cod	Herring River	Herring Pond Dam	Eastham	1.2	41° 49' 22.133" N	69° 59' 18.434" W
Cape Cod Bay	Cape Cod	Herring Brook	Herring Brook Road Control	Eastham	0.4	41° 49' 51.983" N	69° 59' 52.242" W
Cape Cod Bay	Cape Cod	Herring River	Chequesett Road Tide Gate	Wellfleet	0.0	41° 55' 51.991" N	70° 03' 52.150" W
Cape Cod Bay	Cape Cod	Pamet River	Tide Gate	Truro	1.6	41° 59' 37.500" N	70° 03' 01.100" W
Cape Cod Bay	Cape Cod	Pilgrim Lake	Pilgrim Lake Control	Provincetown	0.3	42° 03' 09.800" N	70° 07' 05.900" W
Nantucket Sound	Cape Cod	Pilgrim Lake	Pilgrim Lake Ladder	Orleans	0.4	41° 46' 07.807" N	69° 58' 41.848" W
Nantucket Sound	Cape Cod	Muddy Creek	None	Chatham/Harwich	1.6	N/A	N/A
Nantucket Sound	Cape Cod	Stillwater Pond	Stillwater Pond Fishway	Chatham	0.1	41° 42' 20.079" N	69° 59' 05.091" W
Nantucket Sound	Cape Cod	Frost Fish Creek	Frost Fish Creek Trail Culvert	Chatham	0.3	41° 42' 07.328" N	69° 58' 13.451" W
Nantucket Sound	Cape Cod	Red River	Skinequit Pond Fishway	Harwich	0.3	41° 40' 19.048" N	70° 02' 38.001" W
Nantucket Sound	Cape Cod	Andrews River	None	Harwich	1.8	N/A	N/A
Nantucket Sound	Cape Cod	Herring River	West Reservoir Dam	Harwich	3.9	41° 40' 55.442" N	70° 07' 19.680" W
Nantucket Sound	Cape Cod	Swan Pond River	None	Dennis	2.4	N/A	N/A
Nantucket Sound	Cape Cod	Fresh Pond Tributary	None	Dennis	0.7	N/A	N/A
Nantucket Sound	Cape Cod	Weir Creek	None	Dennis	2.1	N/A	N/A
Nantucket Sound	Cape Cod	Bass River	North Dennis Road	Yarmouth	5.5	41° 42' 18.044" N	70° 11' 38.095" W
Nantucket Sound	Cape Cod	Parkers River	Seine (Swan) Pond Inlet	Yarmouth	2.0	41° 39' 37.904" N	70° 12' 36.426" W
Nantucket Sound	Cape Cod	Town Brook	Mill Pond Fishway	W. Yarmouth	0.0	41° 39' 30.272" N	70° 15' 36.784" W
Nantucket Sound	Cape Cod	Mill Creek	Mill Pond Dam (Baxter Grist Mill)	W. Yarmouth	0.9	41° 39' 27.290" N	70° 15' 40.069" W
Nantucket Sound	Cape Cod	Stewarts Creek	Aunt Betty's Pond Control	Barnstable	1.2	41° 38' 56.554" N	70° 17' 42.533" W
Nantucket Sound	Cape Cod	Halls Creek	Marchant Mill Road Culvert	Barnstable	1.0	41° 38' 06.799" N	70° 18' 32.994" W
Nantucket Sound	Cape Cod	Lake Elizabeth	Lake Elizabeth Dam	Barnstable	2.2	41° 38' 16.016" N	70° 20' 01.623" W
Nantucket Sound	Cape Cod	Centerville River	Wequaquet Lake Control	Barnstable	1.6	41° 39' 36.635" N	70° 20' 05.489" W
Nantucket Sound	Cape Cod	Bumps River	Bumps River Road Culvert	Barnstable	1.1	41° 38' 54.131" N	70° 21' 46.355" W
Nantucket Sound	Cape Cod	Marstons Mills River	Route 28 Stream Baffles	Barnstable	0.9	41° 39' 01.576" N	70° 24' 51.657" W
Nantucket Sound	Cape Cod	Little River	Old Post Road Culvert	Barnstable	0.3	41° 37' 35.627" N	70° 25' 35.224" W
Nantucket Sound	Cape Cod	Rushy Marsh Pond	Rushy Marsh Pond Culvert	Barnstable	0.0	41° 35' 57.796" N	70° 26' 32.610" W
Nantucket Sound	Cape Cod	Santuit River	Mill Road	Mashpee	0.8	41° 37' 40.001" N	70° 27' 03.046" W
Nantucket Sound	Cape Cod	Mashpee River	Bog Sluice DS Washburn Pond	Mashpee	4.2	41° 38' 41.724" N	70° 29' 01.582" W
Vineyard Sound	Cape Cod	Quashnet River	Route 28	Falmouth	1.3	41° 35' 26.065" N	70° 30' 30.019" W

Region	Watershed	Stream	Boundary	City/Town	River Mile	GPS Latitude	GPS Longitude
Vineyard Sound	Cape Cod	Childs River	Barrows Road	Falmouth	0.4	41° 35' 07.034" N	70° 31' 35.095" W
Vineyard Sound	Cape Cod	Mill Pond/Green Pond	Mill Pond Dam	Falmouth	2.2	41° 34' 44.117" N	70° 33' 49.510" W
Vineyard Sound	Cape Cod	Flax Pond (Coonamesett R.)	John Parker Road Culvert	Falmouth	0.1	41° 35' 09.769" N	70° 34' 17.747" W
Vineyard Sound	Cape Cod	Coonamesett River	John Parker Road Fishway	Falmouth	0.3	41° 34' 54.260" N	70° 34' 24.354" W
Vineyard Sound	Cape Cod	Little Pond	None	Falmouth	0.1	N/A	N/A
Vineyard Sound	Cape Cod	Fresh River	Shivericks Pond Dam	Falmouth	1.0	41° 33' 13.623" N	70° 37' 00.897" W
Vineyard Sound	Cape Cod	Salt Pond	None	Falmouth	0.1	N/A	N/A
Vineyard Sound	Cape Cod	Trunk River	Oyster Pond Control	Falmouth	0.2	41° 32' 13.330" N	70° 38' 24.047" W
Buzzards Bay	Cape Cod	Herring Brook	Herring Brook Dam	Falmouth	0.6	41° 37' 24.337" N	70° 37' 45.959" W
Buzzards Bay	Cape Cod	Wild Harbor River	Dam Pond Culvert	Falmouth	0.8	41° 38' 02.436" N	70° 37' 56.091" W
Buzzards Bay	Cape Cod	Ceadr Lake Ditch	Bay Road Culvert	Falmouth	0.3	41° 38' 56.061" N	70° 37' 35.525" W
Buzzards Bay	Cape Cod	Red Brook	Red Brook Conrail Culvert	Bourne	0.0	41° 40' 36.534" N	70° 36' 47.367" W
Buzzards Bay	Cape Cod	Pocasset River	Shop Pond Dam	Bourne	1.0	41° 41' 48.022" N	70° 36' 18.048" W
Islands	Martha's Vineyard	Lagoon Pond	Richard Madieras Fishway	Tisbury/Oak Bluffs	2.2	41° 25' 47.137" N	70° 35' 59.186" W
Islands	Martha's Vineyard	Farm Pond	None	Oak Bluffs	0.0	N/A	N/A
Islands	Martha's Vineyard	Sengekontacket Pond	Sengekontacket Development	Oak Bluffs/Edgartown	1.6	41° 25' 05.720" N	70° 34' 23.528" W
Islands	Martha's Vineyard	Trapps Pond	None	Edgartown	0.0	N/A	N/A
Islands	Martha's Vineyard	Mattakeset Herring Creek	None	Edgartown	1.2	N/A	N/A
Islands	Martha's Vineyard	Edgartown Great Pond	Edgartown Great Pond Barrier	Edgartown	0.0	N/A	N/A
Islands	Martha's Vineyard	Jobs Neck Pond	None	Edgartown	0.0	N/A	N/A
Islands	Martha's Vineyard	Oyster Pond	None	Edgartown	0.0	N/A	N/A
Islands	Martha's Vineyard	Tisbury Great Pond	None	Chilmark/W. Tisbury	0.0	N/A	N/A
Islands	Martha's Vineyard	Mill Brook	Outlet to Tisbury Great Pond	Chilmark	0.0	41° 22' 45.046" N	70° 40' 12.052" W
Islands	Martha's Vineyard	Fulling Mill Brook	Tributary upstream of unamed pond	Chilmark	0.0	41° 20' 40.067" N	70° 43' 08.012" W
Islands	Martha's Vineyard	Tiasquam River	Looks Pond Dam	Chilmark/W. Tisbury	0.3	41° 22' 40.814" N	70° 40' 44.601" W
Islands	Martha's Vineyard	Black Point Pond	None	Chilmark	0.2	N/A	N/A
Islands	Martha's Vineyard	Chilmark Pond	None	Chilmark	0.0	N/A	N/A
Islands	Martha's Vineyard	Roaring Brook	Mouth	Chilmark	0.0	41° 22' 41.082" N	70° 44' 39.062" W
Islands	Martha's Vineyard	Gay Head Herring Creek	None	Chilmark/Aquinnah	0.3	N/A	N/A
Islands	Martha's Vineyard	James Pond	None	W. Tisbury	0.3	N/A	N/A
Islands	Martha's Vineyard	Lake Tashmoo	Old Water Supply Pond	Tisbury/V. Haven	0.1	41° 26' 55.199" N	70° 37' 20.101" W
Islands	Nantucket	Sesechacha Pond	Barrier Beach	Nantucket	0.0	41° 18' 03.686" N	69° 58' 31.904" W
Islands	Nantucket	Folgers Marsh	None	Nantucket	0.8	N/A	N/A
Islands	Nantucket	Hither Creek/Long Pond	None	Nantucket	2.2	N/A	N/A
Islands	Nantucket	Hummock Pond	Barrier Beach	Nantucket	0.0	41° 15' 18.353" N	70° 09' 50.602" W
Islands	Nantucket	Miacomet Pond	Barrier Beach	Nantucket	0.0	41° 14' 36.516" N	70° 07' 05.219" W
SE Mass	Buzzards Bay	Bourne Pond Brook	Bourne Pond Outlet	Bourne	0.4	41° 44' 56.681" N	70° 35' 53.879" W
SE Mass	Buzzards Bay	Red Brook	Route 25 Stream Baffle	Wareham	1.4	41° 46' 34.002" N	70° 37' 50.599" W
SE Mass	Buzzards Bay	Gibbs Brook	Gibbs Brook Culvert	Wareham	0.1	41° 45' 20.484" N	70° 39' 12.952" W
SE Mass	Buzzards Bay	Agawam River	Mill Pond Dam	Wareham	2.9	41° 45' 44.416" N	70° 40' 33.584" W

Region	Watershed	Stream	Boundary	City/Town	River Mile	GPS Latitude	GPS Longitude
SE Mass	Buzzards Bay	Wankinco River	Parker Mills Dam	Wareham	0.7	41° 46' 01.789" N	70° 43' 19.891" W
SE Mass	Buzzards Bay	Weweantic River	Horseshoe Pond Dam	Wareham	4.3	41° 45' 55.047" N	70° 44' 51.047" W
SE Mass	Buzzards Bay	Sippican River	Hathaway Pond Dam	Marion	3.6	41° 44' 02.360" N	70° 47' 39.373" W
SE Mass	Buzzards Bay	Tinkham Pond	Tinkham Pond Control	Mattapoisett	1.2	41° 40' 56.092" N	70° 51' 23.814" W
SE Mass	Buzzards Bay	Mattapoisett River	Route 6 Crossing	Mattapoisett	0.7	41° 39' 25.533" N	70° 50' 03.390" W
SE Mass	Buzzards Bay	Acushnet River	Main Street	New Bedford	4.0	41° 40' 54.055" N	70° 55' 08.038" W
SE Mass	Buzzards Bay	Buttonwood Brook	Buttonwood Pond Park Dam	New Bedford	2.0	41° 37' 55.852" N	70° 57' 13.607" W
SE Mass	Buzzards Bay	Paskamanset/Slocum River	Russells Mills Pond Dam	Dartmouth	0.0	41° 34' 16.661" N	71° 00' 16.430" W
SE Mass	Buzzards Bay	Westport River - East Branch	Old County Road	Westport	8.0	41° 37' 15.050" N	71° 03' 35.020" W
SE Mass	Buzzards Bay	Westport River - West Branch	Gray's Mill Pond Dam	Adamsville, RI	3.4	41° 33' 20.989" N	71° 07' 35.801" W
SE Mass	Buzzards Bay	Cockeast Pond	Cockeast Pond Outlet	Westport	0.1	41° 30' 35.131" N	71° 05' 53.262" W
SE Mass	Buzzards Bay	Richmond Pond	None	Westport	1.6	41° 30' 24.487" N	71° 06' 50.392" W
SE Mass	Taunton	Taunton River	Threemile River	Dighton	10.5	41° 51' 14.032" N	71° 06' 32.044" W
SE Mass	Taunton	Labor in Vain Brook	Somerset Reservoir Outlet	Somerset	0.9	41° 46' 38.295" N	71° 08' 29.843" W
SE Mass	Taunton	Assonet River	Tisdale Pond Dam	Freetown	3.6	41° 47' 45.526" N	71° 03' 56.750" W
SE Mass	Taunton	Rattlesnake Brook	Bleachery Reservoir Outlet	Freetown	0.1	41° 46' 50.976" N	71° 05' 11.744" W
SE Mass	Taunton	Muddy Cove Brook	Elm Street	Dighton	0.5	41° 48' 48.080" N	71° 07' 42.086" W
SE Mass	Taunton	Segreganset River	Unnamed Dam	Dighton	0.9	41° 49' 36.972" N	71° 07' 40.685" W
SE Mass	Taunton	Three Mile River	Dam below Harodite Factory	Dighton	1.1	41° 51' 46.323" N	71° 07' 21.375" W
SE Mass	Taunton	Berkley Street Tributary	Unnamed Dam	Taunton	0.3	41° 52' 30.784" N	71° 05' 21.510" W
SE Mass	Taunton	Oakland Mill (Brickyard) Pond	Oakland Mill Ponds Culvert	Taunton	0.3	41° 53' 20.012" N	71° 04' 50.273" W
SE Mass	Taunton	Mill River	Site of former Hopewell Mills Dam	Taunton	2.4	41° 54' 54.381" N	71° 05' 49.104" W
SE Mass	Narragansett Bay	Lewin Brook	Swansea Print Works Dam	Swansea	0.1	41° 44' 43.054" N	71° 11' 31.095" W
SE Mass	Narragansett Bay	Lee River	Swan Finishing Dam	Swansea	3.0	41° 44' 43.847" N	71° 11' 32.530" W
SE Mass	Narragansett Bay	Cole River	Route 6 Dam	Swansea	2.5	41° 44' 49.723" N	71° 12' 10.208" W
SE Mass	Narragansett Bay	Rocky Run	None	Swansea/Rehoboth	8.6	N/A	N/A
SE Mass	Narragansett Bay	Palmer River	Shad Factory Pond Dam	Rehoboth	7.7	41° 48' 32.204" N	71° 16' 43.526" W
SE Mass	Narragansett Bay	Runnins River	Mobil Dam	E. Providence, RI	0.7	41° 47' 00.145" N	71° 19' 48.546" W

Appendix A.3. Fish Kill Investigation Report Form

1. **Date:**	2. **Arrival Time:** **Departure Time:**	3. **Waterbody Location:**	4. **Person reporting:** Name: Phone: Address: Affiliation:

5. **# of fish Killed**: _____ Incident Size: Minor <100 □ Moderate 100-1000 □ Major >1000 □	6. **Dimensions of fish kill**: __________ by __________	7. **Fish Species Affected:**	**Fish Size**
		1. ______________________	Same □ Different □ Range ____ to ____ in.
		2. ______________________	Same □ Different □ Range ____ to ____ in.
		3. ______________________	Same □ Different □ Range ____ to ____ in.
		4. ______________________	Same □ Different □ Range ____ to ____ in
8. **Fish Species Not Affected**	9. **Weather**	5. ______________________	Same □ Different □ Range ____ to ____ in.
____________________	Temp (F)	6. ______________________	Same □ Different □ Range ____ to ____ in.
____________________	Cloud Cover (%)	7a. **Other Species Affected:**	
____________________	Precipitation (%)	1, ______________________	Dead □ Dying □ Lethargic □ Live □
____________________	Wind Speed (mph)	2. ______________________	Dead □ Dying □ Lethargic □ Live □
____________________	Wind direction	3. ______________________	Dead □ Dying □ Lethargic □ Live □
		4. ______________________	Dead □ Dying □ Lethargic □ Live □

10. **Water Quality:**	11. **Water Condition:**	12. **Fish Condition:**			
Temp (C): __________		Dying □	Discoloration □	Increased respiration □	Emaciated □
pH: __________	Turbid □	Gills flared □	Odd fin position □	Eyes sunken in □	Spasms, convulsions □
DO: __________	Sediment Loading □	Red/pink gills □	Swimming at surface □	Eyes bulging □	Erratic Swimming □
Conductivity: __________	Colored: __________ □	Gill clubbing □	Equilibrium loss □	Bloated □	Lethargy □
Salinity: __________	Odor: __________ □	Excessive mucus □	Trying to get out of water □	Mouth agape □	Hemorrhaging □
Chlorine: __________	Tidal Stage: __________	Lesions □		Hypersensitivity □	Spine curved □
Alkalinity: __________	SAV/ macroalgae ______ □	Other __________		Run samples for: __________	

13. **Symptoms/Conditions**	**Possible Cause**	**Possible Source**	**Source present?**	
• Fish coming to surface gulping for air □ • Low dissolved oxygen □	Oxygen depletion	Sewage Treatment Plan	Yes □	No □
		Livestock Feedlot	Yes □	No □
		Irrigation/De-icing Runoff	Yes □	No □
		Decaying Plant Matter	Yes □	No □
		Dying Algal Bloom	Yes □	No □
• Fish coming to surface gulping for air □ • Adequate dissolved oxygen □	Early oxygen depletion with slow re-oxygenation	Ammonia Chemicals	Yes □	No □
		Livestock Feedlot	Yes □	No □
• Fish swimming erratically □ • Fish moving upstream to avoid something in water □	Chemical pollution	Heavy Metal Plant	Yes □	No □
		Chemical Waste Facility	Yes □	No □
		Sewage Treatment Plant	Yes □	No □
• Fish dying or dead after heavy rain □	Pesticide, herbicide washed out/runoff	Farms, Crop fields	Yes □	No □
		Aerial Crop Sprayer	Yes □	No □
		Man/mechanical Sprayer	Yes □	No □
• Fish coming to surface gulping for air □	Oxygen depletion	Dredging/ Marina activity	Yes □	No □
• Low pH □ Good clarity □ Orange Discoloration □	Acid	Coal/Strip Mining	Yes □	No □
• Fish dying below a dam or industrial plant □	Turbines or thermal shock	Heated water	Yes □	No □
• Kill restricted to one species or size class □	Spawning stress, disease	Pathogens, WQ poor	Yes □	No □

14. **Documentation and Samples**: Photos taken □ Water samples □ Number: __________ Sent to: __________________ Tested For: ________
Fish Samples □ Number: __________ Sent to: __________________ Tested For: ________

Additional Comments:

Appendix 5A.4. Fish-Kill Subsample Data Form.

Date: _______ Time: Start _______ Finish _______ Name of Investigator(s): __________________

Location/Waterbody: ______________________________ Transect/Segment # _______

Species	Sex	TL (mm)	Wgt (g)	Species	Sex	TL (mm)	Wgt (g)

Comments: