

Massachusetts Estuaries Project

Benthic Monitoring

Laboratory Standard Operating Procedures

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Laboratory Standard Operating Procedures

for

MassDEP Massachusetts Estuaries Project

Benthic Monitoring

Submitted to

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Massachusetts Estuaries Project
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I. General Information

1.0 Introduction

The Massachusetts Department of Environmental Protection (MassDEP) established the Massachusetts Estuaries Project (MEP) to monitor and protect estuarine ecosystems in southeastern Massachusetts embayments. MEP's goal is to assess the conditions of these embayments and to develop critical site-specific nitrogen thresholds that could be used as a management tool by communities to identify needed corrective and protective measures for both now and in the future.

Benthic infaunal communities are a good indicator of embayment conditions and are used to assess the level of habitat health from healthy (low organic matter, high D.O.) to highly stressed (high organic matter, low D.O.). Communities in benthic assemblages respond to a variety of stressors in different ways allowing the type of stress affecting the assemblage to be identified. As many macroinvertebrates have relatively long life cycles of a year or more and are relatively immobile, the structure of the assemblage is a response to past and/or present conditions (Howes et al. 2003, US EPA 2015). MEP uses the approach, which is accepted by the regulatory community, that the pollution tolerance of individual species allows their use as indicators in relation to pollution effects on estuarine and marine habitats.

The objectives of the MEP benthic monitoring program are to assess embayment ecological health and to determine if long term changes are occurring in southeastern Massachusetts estuaries that may indicate stress from nutrients and other factors including invasive species and climate change. Additional information can be found in the MEP Benthic Monitoring Quality Assurance Project Plan (QAPP; Rutecki and Nestler 2019).

This document constitutes the Standard Operating Procedures (SOP) manual for the laboratory tasks of benthic monitoring for the Massachusetts Estuaries Project. The goals of this SOP are to (1) provide sufficiently detailed instructions to enable laboratory technicians to follow consistent and technically valid protocols, and to (2) record the laboratory results from these surveys in a consistent manner.

2.0 Technical Approach

MEP benthic monitoring will include laboratory analyses to support sediment and benthic macroinvertebrate community characterization. This SOP manual contains detailed procedures for analyses of the following types of benthic samples:

- Benthic Macrofauna
 - Soft bottom samples using a Van Veen grab sampler
 - Hard bottom/riprap samples using a suction sampler

- Soft Bottom Sediment Analysis
 - Grain size analysis
 - Total Organic Carbon (TOC)
- Underwater Digital Still Images and/or Video
- Soft Bottom Sediment Profile Imaging (SPI)

3.0 References

- Howes, B.L., R. Samimy, and B. Dudley. 2003. Massachusetts Estuaries Project. Site-Specific Nitrogen Thresholds for Southeastern Massachusetts Embayments: Critical Indicators. Interim Report. Prepared for Massachusetts Department of Environmental Protection. 25 pp.
- Rutecki, D. and E. Nestler. 2019. Massachusetts Estuaries Project, Benthic Monitoring Quality Assurance Project Plan (QAPP). Draft Revision 1. Prepared by Normandeau Associates, Inc., Falmouth, MA. March 2019. 105 pp.
- US EPA (U.S. Environmental Protection Agency). 2015. National Coastal Condition Assessment: Field Operations Manual. Version 1.0, May 2015. EPA-841-R-14-007. U.S. Environmental Protection Agency, Office of Water, Washington, DC. 166 pp.

II. Laboratory Standard Operating Procedures for Benthic Macrofauna Samples

Samples are preserved in the field with formalin and delivered or shipped to a contracted laboratory. The following section describes the laboratory procedure for sorting and taxonomic identification of macrobenthic organisms collected from MEP soft-bottom infauna surveys using a Van Veen grab or hard bottom/riprap destructive sampling (epifauna) using a suction sampler. These methods are consistent with the laboratory procedures outlined in the National Coastal Condition Assessment (NCCA) 2015 Laboratory Operations Manual (US EPA 2016). For additional program details refer to the MEP Benthic Monitoring QAPP (Rutecki and Nestler 2019).

1.0 Health and Safety

In addition to the laboratory's requirements, persons using this SOP must abide by the following health and safety procedures:

1. Wear proper personal protection clothing and equipment (e.g. lab coat, gloves, and protective eyewear/goggles).
2. When working with potentially hazardous chemicals (e.g. formalin, reagent alcohol, Rose Bengal) or biological agents (benthic organisms and sediments), laboratory personnel must follow all manufacturer's Safety Data Sheet recommendations, and avoid inhalation, skin contact, eye contact, or ingestion. If skin contact occurs, remove clothing immediately and rinse thoroughly. Wash the affected skin areas thoroughly with large amounts of soap and water. When working with formalin, laboratory personnel must follow the OSHA Formaldehyde Standard (29 CFR 1910.1048).

2.0 Laboratory Equipment

2.1 Sample Preparation - Sorting

The following equipment and materials are required for sample preparation, subsampling, sorting, and taxonomic identifications:

- U.S. 35 sieve (500 μm or 0.5 mm) for soft-bottom infaunal samples
- U.S. 18 sieve (1000 μm or 1.0 mm) for hard-bottom/riprap destructive samples
- Round buckets
- Standardized, or gridded screen - 40 Mesh (380- μm openings, T304 stainless steel wire, 34GA (0.010"))
- 6-cm scoop
- White or clear plastic or enamel pan (6" x 9") for sorting
- Teaspoon
- Permanent ink pen (e.g. Pigma Micron® pen, Sharpie)
- Pencil or alcohol resistant ink pen

- Dropper
- Fine-tipped forceps (watchmaker type, straight and curved)
- Vials with caps or stoppers
- Sample labels for vials
- Reagent alcohol (5% methanol, 5% isopropanol, 85% ethanol)
- Stereo zoom microscope (6-10X magnification or greater)

2.2 Sample Preparation - Taxonomy Identification

The following equipment is required for benthic macroinvertebrate taxonomic identification:

- Stereo dissecting microscope with fiber optics light source (50-60X magnification)
- Compound microscope (10, 40, and 100X objectives, with phase-contrast capability)
- Digital camera with high resolution capability mounted on a microscope (optional)
- Petri dishes
- Permanent ink pen (e.g Pigma Micron® pen, Sharpie)
- Pencil or alcohol resistant ink pen
- Dropper
- Fine-tipped forceps (watchmaker type, straight and curved)
- Vials with caps or stoppers
- Sample labels for vials
- Reagent alcohol in a plastic wash bottle
- Taxonomic Bench Sheet
- Hand tally counter
- Taxonomic keys (For example, Smith 1964, Gosner 1971, Bousfield 1973, Abbott 1974, Fauchald 1977, and Pollock 1998).

3.0 Sample Receipt

The laboratory procedure for benthic samples begins with the receipt of the samples at the subcontracted laboratory.

1. Record receipt of samples and sign the Chain of Custody (COC) form (Figure 1).
2. Inspect each jar THE SAME DAY THEY ARE RECEIVED:
 - a. Add 10% Formalin to the jar, if necessary (i.e., to cover the contents completely).
 - b. Verify that the date collected, site identification, and sample number on the label also appear on the Chain of Custody form in the shipment.

- c. Notify the Project Manager if any jars were broken and/or there are discrepancies between the custody form and jars.
3. Store the sample containers at room temperature until sorting begins. Replace the 10% buffered formalin with reagent alcohol within 7 days of collection for better preservation of the organisms.
4. To facilitate the sorting process, all samples will be stained with Rose Bengal. Add Rose Bengal to the reagent alcohol to the point of saturation. Samples should be stained at least overnight but no longer than 48 hours before sorting the infaunal samples to avoid over-staining.
5. Maintain the Chain of Custody form with the samples; it will be needed if the samples are transported to any other location (e.g., for taxonomic identification, external quality control (QC) evaluation).

4.0 Sample Preparation - Sorting

This section describes the steps for the sorter in preparing the sample and picking organisms.

1. Remove the lid from the sample container and remove the internal sample label.
2. Carefully decant the reagent alcohol from the sample container by pouring the fluid through a 0.5 mm or 1.0 mm (selected based on the sample type being sorted) sieve into a separate container. Inspect the mesh of the sieve for any organisms and return any organisms found to the sample container so they can be included in the sample sort process.
3. Remove sieved organisms from the sample container and place into a sorting tray.
4. Sort all samples under a minimum of 6x dissecting microscope. Remove the macroinvertebrates from the detritus with forceps. In general, do not remove:
 - Empty snail or bivalve shells
 - Organisms of water surface-dwelling or strict water column arthropod taxa, and meiofauna.
 - Incidentally-collected terrestrial taxa.
 - Fragments such as legs, antennae, gills, wings, or tails.
 - For Oligochaeta, attempt to remove only whole organisms or fragments that include the head. Do not remove fragments without the head.
 - In case of uncertainties, place the organism in the sort vial for the taxonomist to make the final determination.
5. Place picked organisms of the same type into a single set of jars and vials containing reagent alcohol.
6. Remove the remaining material left on the sorting pan (i.e. material such as sticks, organic debris) and place it in a separate container with preservative (reagent alcohol). Label the container "Picked," on both internal and external labels.

7. Label the vials and jars of sorted organisms and material with an external label using a permanent ink pen. Internal sample labels should be made of cotton rag paper or an acceptable substitute and written with pencil or alcohol resistant ink pen.
8. Retain the vials and materials for the time period specified in Section 9.0.
9. Thoroughly clean all sample preparation and sorting equipment and make sure all equipment is free of organisms prior to sorting the next sample.

5.0 Taxonomic Identification

The taxonomist performs the following steps in identifying the benthic macroinvertebrates:

1. Upon receipt of a set of sample vials from the sorter:
 - a. Compare all site identification codes and sample numbers on the form with those entered on the labels of samples, and resolve any discrepancies with the sorter.
 - b. Determine if any vials are broken. For any broken vial, attempt to recover as much of the sample as possible. Describe the damage on the Taxonomic Bench Sheet.
 - c. Maintain the Chain of Custody form with the sample vials; it will be needed to return/store them.
2. Empty one sample vial at a time into a small Petri dish. Add reagent alcohol to keep the organisms covered. Remove the internal sample label and complete the top portion of a Taxonomic Bench Sheet, using the information from the label.
3. View the sample to ensure that all necessary diagnostic characters have been observed, according to the taxonomic key or other literature.
4. Identify organisms to the lowest practical taxonomic level (species is the target for all organisms with the exception of meiofauna, (due to being smaller than 0.5 mm). Additional exceptions include Oligochaeta (Class) and Chironomidae (Family) in samples from marine, polyhaline and mesohaline regions. If a laboratory or individual taxonomist is having trouble reaching the species level for a taxonomic group but not for an individual organism which might be damaged or otherwise difficult to identify, the lab must contact the project lead for guidance. Add any necessary data qualifiers.
5. Record the identifications. For example, using the Taxonomic Bench Sheet, record the identification in the Column labeled "taxon." Enter the number of larvae, pupae, and adults, or total count (e.g. mollusks), if appropriate life history column does not apply, of each taxon under the appropriate columns.
 - a. Refer to either website to check the scientific name to be sure that there have not been any name changes: 1) <https://www.itis.gov/> or 2) <http://www.marinespecies.org/aphia.php?p=search>

- b. If the target taxonomic level cannot be achieved due to immature or damaged organisms this should be noted.
- c. If damaged organisms can be identified, they are counted ONLY if the:
 - 1) Fragment includes the head, and, in the case of arthropods, the thorax;
 - 2) Oligochaetes have a sufficient number of segments in the head;
 - 3) Mollusk shell (bivalve or gastropod) is occupied by an organism;
 - 4) Organism is the sole representative of a taxon in the sample.
- d. If a unique taxon is determined for which the appropriate taxonomic level is not available in the literature and there are other taxa in that taxonomic level:
 - 1) Provide good quality digital photographs of the organism to outside experts for identification; and
 - 2) Include the tentative identification in the database with a data qualifier so that these organisms can be distinguished from other organisms in the data analysis.
 - 3) When the outside expert identifies the organism, update the database with the correct identification.
6. Compare taxa names from the taxa list provided by the project manager to the names used for the identifications. Check the non-matches and correct them.
7. Complete the identification by entering the totals for each developmental stage and the total number of each taxon in the cells at the bottom of the sheet. Cross-check to be sure the totals were summed correctly.
8. Return the identified organisms to the original sample vial, fill with reagent alcohol, and cap tightly.
9. Return or store the samples according to laboratory protocols and requirements.
10. Verify that all required data has been recorded by the taxonomist or QC personnel. If the results were recorded on paper, provide the Taxonomic Bench Sheet to data entry staff.

6.0 Data Entry

All data generated by taxonomic identification will be manually read from the instrument display (optical field of a microscope) and entered directly into an electronic format (e.g., Excel spreadsheet), or entered into laboratory forms or data sheets, and then manually entered into an electronic format. All manually entered data will receive 100% verification or will be entered and checked using double data entry. Standardized codes and qualifiers help to ensure consistency over time in a benthic monitoring program. Tables 1 and 2 identify the required data codes that the contracted laboratory must provide to the Project Manager for benthic macrofauna samples.

Data reduction is the process of converting raw numbers (e.g., numbers of organisms per replicate) into data that can be displayed graphically, summarized in tables, or compared statistically for differences between mean values for sampling stations or times.

Macrofauna data analysis requires that some data be derived from the raw numbers for the Synthesis Report. All data reduction will be performed electronically, either by the instrument software or in a spreadsheet, and will be validated according to procedures described in the MEP Benthic Monitoring QAPP Section D (Rutecki and Nestler 2019).

Prior to the release of any data from the contracted laboratory, the data will be reviewed and approved by laboratory personnel. The review will consist of a tiered approach that will include reviews by the person performing the work, by a qualified peer, and by supervisory and/or QA personnel.

Table 1. Benthic macrofaunal and sediment sample data codes. Data codes are from NCCA 2015 (US EPA 2016).

Field	Format	Description	
LAB NAME	Character	Name of lab	
DATE RECEIVED	MMDDYY	Date sample was received by lab	
SITE ID	Character	Site identification code as used on sample label	
SAMPLE_NUMBER	Numeric	Sample number as used on field sheet (on sample label)	
DATE COLLECTED	MMDDYY	Date sample was taken	
CONDITION_CODE	Character	Condition codes describing the condition of the sample upon arrival at the laboratory.	
		Flag	Definition
		OK	Sample is in good condition
		C	Sample container is cracked
		ML	Sample label is missing
		NP	Not enough preservative used
		Q	Other quality concerns, not identified above (explain in COND_COMMENTS)
COND_COMMENTS	Character	Explanation for Q FLAG (if needed)	

Table 2. Benthic macrofaunal taxonomic identification data codes. Data codes are from NCCA 2015 (US EPA 2016).

Field	Format	Description	
LAB NAME	Character	Name of lab	
DATE RECEIVED	MMDDYY	Date sample was received by lab	
SITE ID	Character	Site identification code as used on sample label	
SAMPLE_NUMBER	Numeric	Sample number as used on field sheet (on sample label)	
DATE COLLECTED	MMDDYY	Date sample was taken	
DATE TAXON	MMDDYY	Date that the taxonomist started identifying organisms in the sample	
CONDITION_CODE	Character	Condition codes describing the condition of the sample upon arrival at the laboratory.	
FAMILY	Character	Taxonomic family	
GENUS	Character	Taxonomic genus	
SPECIES	Character	Taxonomic species	
TSN	Numeric	Taxonomic Serial Number is a unique and persistent identifier for a scientific name in the Integrated Taxonomic Information System (ITIS). If taxon is not in this list, provide citation for reference used to identify organism in CITATION field	
TAXA NAME	Character	Complete taxon name	
ABUNDANCE TOTAL	Numeric	Total number of individuals	
DISTINCT	Character	Distinct taxa in sample (y/n)	
CITATION	Character	Citation for reference used to identify organism, if taxon not present in ITIS.	
QA FLAG (if appropriate)	Character	QA/QC flag (lab may use its own flags, if defined in QA_COMMENTS field)	
		Flag	Definition
		DD	Damaged Organism, poor condition or fragments
		NP	Not enough preservative used
		UN	Unknown. Identification is tentative. Organism has been sent to expert taxonomist for definitive identification.
		NT	Not able to meet target level for identification (may be used with other codes, or explain in QA_COMMENTS field)
		S	Sample shipping problem (explain in QA_COMMENTS field)
Q	Other quality concerns, not identified above		
COND_COMMENTS	Character	Explanation for Q FLAG (if needed)	
LAB COMMENTS	Character	General laboratory analysis comments	

7.0 Sample and Record Retention

The laboratory shall retain:

1. Macrofauna sample (both archived and processed samples) and sample materials, including vials, slides, and sorting residuals, will be held until acceptance of the Synthesis Report by the Town(s) and MassDEP. These samples can then be disposed of after approval from the Program Manager. Processed samples will be maintained at the laboratory contracted for sorting and identification. Macrofauna sample residues will be held until the data report is accepted by MassDEP, and then may be discarded. Reference collection specimens will be retained by the contracted laboratory until the next survey (3 to 5 years) and then provided to the next designated laboratory. Reference collection specimens will be clearly identified, labeled with the project name and unique identification number. Materials shall be stored in a cool location away from sunlight. The laboratory shall periodically check the reference collection and sample materials for degradation and refill jars and vials with reagent alcohol if necessary.
2. All the project records, including laboratory notebooks and the reference library, will be maintained at least 5 years.

After the stated time periods, the laboratory shall follow its internal protocols for disposal.

8.0 Sample QC

Benthic samples will be checked for QA/QC following the procedure detailed in the MEP Benthic Monitoring QAPP and presented below.

The data quality goals for analysis of benthic macrofauna are (1) all samples be processed, (2) all animals be removed for identification and enumeration, (3) all infaunal animals be counted accurately, (4) the taxonomic identifications be accurate (correct), and (5) the identifications correspond to those used throughout the monitoring program. At least 95 percent of all animals must be removed from a sample to pass the quality control (QC) evaluation.

Sorting

Sorting technicians will remove all organisms from the samples and separate them into major taxonomic groups. All residual material will be labeled and stored for QC analysis. For the QC analysis, samples will be divided into batches of approximately 10 samples. Approximately 10% of the samples from each batch will then be randomly chosen for an independent QC check. Only senior technicians will perform the QC evaluations (A senior technician is defined as having three or more years of sorting experience). Under no circumstances will the same individual who sorted the sample perform the QC evaluation. In most cases, a batch of samples is defined as ten consecutively sorted samples. By definition, at least 95% of all animals must be removed from a sample to pass the QC evaluation (i.e., the percent sorting error must be $\leq 5\%$). The following formula will be used to calculate the percent sorting error for each QC sample:

$$\frac{\text{Number of animals found in QC inspection}}{\text{Total number of animals present in sample}} \times 100 = \text{percent sorting error}$$

If more than 5% of the total organisms in the QC sample have been missed, the sample fails QC evaluation, and the all remaining samples from that batch will be re-sorted. Technicians will be informed of any necessary corrective measures. This procedure will be repeated until the batch of samples passes the QC evaluation. An exception will be made for low abundance samples (a sample with fewer than 60 organisms) that are chosen for the QC evaluation. Any low abundance sample in which three or fewer organisms were missed is considered to pass the sorting QC evaluation even if the percent sorting error is >5%. Samples in which no organisms are present will be excluded from the sorting QC selection process. A record of all sorting QC evaluations will be maintained for each batch.

Identification and Enumeration

The same basic QC principles described in the Sorting section will in general apply to species identifications. At least 10% of the samples will be checked to detect any unacceptable identification and enumeration errors. Only senior taxonomists will perform the QC check. QC samples will be selected in the same manner as described in the Sorting section above. Additionally, the same percent accuracy level will be used to determine if a sample passes the QC evaluation and the same corrective measures will be implemented if a sample fails the QC evaluation. The following formula will be used to calculate the percent taxonomy error for each QC sample:

$$\frac{\text{Total number of taxonomy errors}}{\text{Total number of animals present in sample}} \times 100 = \text{percent taxonomy error}$$

In certain cases it may not be necessary to reprocess the entire batch of samples if only minor corrections are needed (e.g., name changes). When any misidentification is discovered, all previously identified samples containing that taxon will be rechecked. A record of all identification QC evaluations will be maintained.

9.0 Storage

1. Upon completion, the sorted material and the vials of identified animals are boxed for off-site storage.
2. A Storage Label (Figure 2) will be completed and attached to one end of the storage box. The following information is to be provided:
 - a. Project Name, Box Number, and Project Date
 - b. Brief description of the package contents
 - c. Storage Date

- d. Preservative
- e. Manager's Name
3. On the Sample Storage Sheet (Figure 3) an accurate listing of the project name and number, collection date, station/replicate, and description for each sample will be completed. While only one task should be entered on a Sample Storage Sheet, multiple boxes may be included on a Sample Storage Sheet.
4. The original Sample Storage Sheet is placed in the Laboratory Storage Book and a copy placed in the box.
5. Samples are stored in the laboratory's storage facility until the Synthesis Report is approved by the Town(s) and MassDEP. With permission from the Program Manager, following report approval, samples are removed from storage and prepared for sample disposal as described below in Section 10

10.0 Disposal

1. Upon authorization from the Project Manager, samples will be removed from the storage facility and prepared for sample disposal.
2. Sample disposal, the separation of liquids and solids from processed samples, is conducted at the laboratory's ventilated hood by trained individuals.
3. Liquids and solids (including sample residue, glass vials, animals and electrical tape) from sample residue are separated, containerized and labeled following appropriate hazardous waste requirements.
4. The laboratory will adhere to local and state Hazardous Waste Small Generators requirements and hold wastes on site until the subcontracted hazardous waste vendor arrives and removes the wastes. Appropriate documentation, for example copies of the Hazardous Waste Manifest and the Land Ban Form, are filed with the laboratory's manager.

11.0 References

- 29 CFR 1910.1048. Occupational Safety and Health Administration, Department of Labor. Occupational Safety and Health Standards, Toxic and Hazardous Substances. Formaldehyde. Title 29 Code of Federal Regulations. Part 1910.1048.
- Abbott, R.T. 1974. American Seashells. The Marine Mollusca of the Atlantic and Pacific Coasts of North America. Van Nostrand Reinhold Company, New York.
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- Smith, R.I. 1964. Keys to Marine Invertebrates of the Woods Hole Region. Marine Biological Laboratory, Woods Hole, MA.
- US EPA (U.S. Environmental Protection Agency). 2016. National Coastal Condition Assessment 2015: Laboratory Operations Manual. Version 2.1, May 2016. EPA-841-R-14-008. U.S. Environmental Protection Agency, Office of Water, Washington, DC. 214 pp.

Chain of Custody Form

Project Name: _____					Presv.		Parameters				Page of	
Project Number: _____											Method of Shipment:	
Originating Contact: _____												
Originator Location: _____												
Final Destination: _____												
Sampler(s): _____												
Sample		Collection		Containers		Grab.	Comp.					Comments Below:
No.	Identification	Date	Time	No.	Type							
Total												
Relinquished by: (signature)	Received by: (signature)	Relinquished by: (signature)	Received by: (signature)	Relinquished by: (signature)	Received by: (signature)	Relinquished by: (signature)	Received by: (signature)	Relinquished by: (signature)	Received by: (signature)	Relinquished by: (signature)	Received by: (signature)	
Printed Name:	Printed Name:	Printed Name:	Printed Name:	Printed Name:	Printed Name:	Printed Name:	Printed Name:	Printed Name:	Printed Name:	Printed Name:	Printed Name:	
Date:	Date:	Date:	Date:	Date:	Date:	Date:	Date:	Date:	Date:	Date:	Date:	

Figure 1. Example of a Chain-of-Custody Form.

PROJECT NAME:	BOX NUMBER:
PROJECT CODE:	MANAGER:
PROJECT DATE:	STORAGE DATE:
SAMPLE DESCRIPTION:	
SAMPLE INFO: <input type="checkbox"/> RESIDUE <input type="checkbox"/> CONT. <input type="checkbox"/> VIALS	PRESERVATIVE: <input type="checkbox"/> 6% FORM. <input type="checkbox"/> 70% ETOH

BoxLabel.AI_2015

Figure 2. Example of a Storage Label.

Storage Sheet

Project Name: _____ Project Year: _____ Page _____
 Project Code: _____ Project Manager: _____
 Project Task: ICH(IP) _____, ICH(Ent) _____, Benthos _____, Other _____

Collection Dates	Sample Type			Sample Information			Preservative		NAI Box Number	Name/Date		
	Res.	Vial	Cont.	Station	In-house Nr.	6% Form	70% Etoh	Complete		Offsite Storage	Return to Dispose	

Figure 3. Example of a Sample Storage Sheet.

III. Laboratory Standard Operating Procedures for Sediment Analysis

At each sampling site, the Field SOP instructs the crews to collect sediment samples for grain size analysis and total organic carbon (TOC). The field crew then ships the samples on wet ice to a subcontracted laboratory. Once the samples arrive, the laboratory will store the TOC samples in a freezer at -20°C and refrigerate the grain size samples. The holding time for grain size analysis and TOC is 28 days. For additional program details refer to the MEP Benthic Monitoring QAPP (Rutecki and Nestler 2018).

1.0 Health and Safety

The laboratory must require its staff to abide by appropriate health and safety precautions which includes wearing proper personal protection clothing and equipment (e.g. lab coat, protective eyewear, gloves). When working with potentially hazardous chemicals (e.g. weak acid), laboratory personnel must follow all manufacture's Safety Data Sheet recommendations and avoid inhalation, skin contact, eye contact, or ingestion. Laboratory personnel must avoid contacting skin with acid. If skin contact occurs, remove clothing immediately. Wash the affected skin areas thoroughly with large amounts of water.

2.0 Laboratory Equipment

The analytical methods selected for grain size and TOC analysis specify the required equipment.

3.0 Sample Receipt

Upon arrival of the samples, the laboratory must inspect the samples promptly on receipt. As samples arrive, the laboratory must:

1. Record receipt of samples and sign the Chain of Custody (COC) form (Figure 1).
2. Inspect each jar THE SAME DAY THEY ARE RECEIVED:
 - a. Verify that the site identification and sample number on the label also appear on the Chain of Custody form in the shipment.
 - b. Notify the Project Manager if any jars were broken and/or there are discrepancies between the Chain of Custody form and jars.
3. Maintain the Chain of Custody form with the samples; it will be needed if the samples are transported to any other location.
4. Check that each shipping container has arrived undamaged. Check the temperature of one of the samples in the cooler using a thermometer that reads from 21 °C (i.e., room temperature) down to -20 °C or lower (i.e., the expected temperature of frozen samples), or an infra-red (IR) temperature "gun" and record the reading. Field crews ship sediment samples on wet ice; the batch laboratory freezes the sample and ships with dry ice. Record the condition and temperature of the sample in the database.

5. Verify that all that all required data has been recorded by laboratory personnel (Table 3).
6. Transfer the TOC samples to the freezer for storage. Except during processing and analysis stages, the samples must be stored frozen to less than or equal -20 °C and transfer the samples for grain size analysis to the laboratory refrigerator.
7. Notify the Project Manager immediately concerning any problems involving sample integrity, conformity, or inconsistencies as soon as possible following sample receipt and inspection.

Table 3. Sediment analysis sample data codes. Data codes are from NCCA 2015 (US EPA 2016).

Field	Format	Description	
SITE ID	Character	Site identification code as used on sample label	
SAMPLE_NUMBER	Numeric	Sample number as used on chain of custody form (on sample label)	
DATE COLLECTED	MMDDYY	Date sample that the field crew collected the sample	
ANALYSIS_TYPE	Numeric	GRAIN SIZE or TOC	
ARRIVAL_TEMP	Numeric	Temperature of sample upon arrival at the laboratory.	
CONDITION_CODE	Character	Condition codes describing the condition of the sample upon arrival at the laboratory.	
		Flag	Definition
		OK	Sample is in good condition
		C	Sample container is cracked
		L	Sample or container is leaking
		ML	Sample label is missing
Q	Other quality concerns, not identified above (explain in COND_COMMENTS)		
COND_COMMENTS	Character	Explanation for Q FLAG (if needed)	

4.0 Analytical Procedures

The laboratory shall perform analysis of sediment samples to determine grain size and concentrations of TOC. The laboratory will follow all QC/QA procedures for the analytical method being followed. No field-collected QC samples, including field duplicates, or equipment and field blanks for sediment chemistry are required. Adequate sediment is collected for the analytical laboratories to perform the required matrix spike/matrix spike duplicate (MS/MSD) analyses.

4.1 TOC

TOC samples will be sent to an independent laboratory that uses the Lloyd Kahn Method. Results will be reported in units of mg/kg with a method detection limit (MDL) of 0.01% (Kahn 1988). Additional details can be found in Kahn(1988).

4.2 Grain Size Analysis

Grain size can be done by any method that reports the determination as percent silt and meets QA/QC requirements. An example of one method is provided below.

Laboratory Equipment

The following equipment is required for grain size analysis:

- Nest of 6 sieves (2 mm, 1 mm, 500 μ , 250 μ , 125 μ , and 63 μ)
- Two 5 gallon receptacle bucket, on which nested sieves will snugly fit
- Sink with a spray nozzle
- Approximately 4 feet of rubber tubing
- Disposable cupcake tins/baking cups or small similarly sized metal pans
- Drying oven (135 °F)
- Electronic digital scale (0.01 g accuracy)
- 500 ml squirt bottle filled with water
- Spoon or spatula

Procedure

The procedure for grain size analysis is as follows:

1. Pre-weigh 7 pre-labeled cupcake tins (one for each of the sieve's contents plus the final wash water/silty-clay rinse product) and record the weight. For example, weight for tin labeled #1 corresponds to 2 mm sieve, tin #2 corresponds to 1 mm sieve, etc.).
2. Place the nest of 6 sieves in decreasing size, largest, 2 mm sieve on top, and 63 μ sieve on the bottom, over the 5 gallon bucket. Make sure the fit on the bucket is snug such that 100% of the water is retained by the bucket.
3. Place entire contents of the sediment sample into the top of the 6 nested sieves washing all residue from the container using a squirt bottle. If placing the entire sample is causing the sieves to clog, a smaller portion (for example $\frac{1}{4}$ of the sample) can be placed into the top sieve and rinsed until the water has passed through all sieves, then another $\frac{1}{4}$ of the sample placed in and rinsed through. The entire sample will be washed through the sieves, but in smaller increments instead of all at once.
4. Using the spray nozzle on the sink, gently spray the sediment contents in the top sieve until water coming through the sieve is clear. This can be determined by lifting the sieve up at an angle while gently spraying the contents.

5. Transfer contents to the corresponding tin using spoon or spatula and squirt bottle.
6. Repeat steps 2 through 5 for each of the sieves.
7. Residue left in the 5- gallon bucket is the $< 63 \mu$ sized silt and will need to settle out of suspension by sitting undisturbed for 24 hours.
8. Place the cupcake tins into the drying oven set to 135 °F and let sit undisturbed until there is no sign of internal moisture within the residue (approximately 24 hours). Most samples are dry within 24 hours.
9. After the residue in the 5 gallon bucket has settled out for 24 hours, siphon off the clear water using the rubber tubing, stopping before any sediment is sucked up into the tubing. Place the residue into one (or more if needed) pre-labeled and pre-weighed cupcake tins using a squirt bottle. Place into the drying oven until dry.
10. Once all samples are dried, weigh the contents while still in the cupcake tin (0.01 g).
11. Discard contents.
12. Calculate the weight for each grain size by subtracting the pan weight from the total weight, and record on data sheet under each of the size categories.

Grain size will be classified following the Coastal and Marine Ecological Classification Standard (CMECS) sediment grain size descriptors (FGDC 2012) and reported as a percentage by weight in five categories as follows:

- Very coarse sand = sum of 2 mm and 1 mm sieve material
- Coarse sand = 500 μ to < 1 mm (0.5 to < 1 mm)
- Medium sand = 250 μ to $< 500 \mu$ (0.25 to < 0.5 mm)
- Fine sand = 125 μ to $< 250 \mu$ (0.125 to < 0.25 mm)
- Very fine sand = 63 μ to $< 125 \mu$ (0.0625 to < 0.125 mm)
- Silt = $< 63 \mu$ (< 0.0625 mm)

5.0 Data Entry

All data generated by the laboratory will be either electronically transferred from the instrument or manually read from the instrument display (e.g. digital scale display) and entered directly into an electronic format, or into laboratory forms and then manually entered into an electronic format. All manually entered data will receive 100% verification or will be entered and checked using double data entry. Standardized codes and qualifiers help to ensure consistency over time in a benthic monitoring program. Tables 3 and 4 identify the required data codes that the laboratory must provide to the Project Manager for sediment samples.

Prior to the release of any data from the contracted laboratory, the data will be reviewed and approved by laboratory personnel. The review will consist of a tiered approach that will include reviews by the person performing the work, by a qualified peer, and by supervisory and/or QA personnel.

Table 4. Sediment grain size and TOC analysis data codes. Data codes are from NCCA 2015 (US EPA 2016).

Field	Format	Description	
SITE ID	Character	Site identification code as used on chain of custody form (on sample label)	
SAMPLE_NUMBER	Numeric	Sample number as used on chain of custody form (on sample label)	
DATE COLLECTED	MMDDYY	Date sample that the field crew collected the sample	
ANALYSIS_TYPE	Character	GRAIN SIZE or TOC	
ARRIVAL_TEMP	Numeric	Temperature of sample upon arrival at the laboratory.	
CONDITION_CODE	Character	Condition codes describing the condition of the sample upon arrival at the laboratory.	
		Flag	Definition
		OK	Sample is in good condition
		C	Sample container is cracked
		L	Sample or container is leaking
		ML	Sample label is missing
		NP	Not enough preservative used
		VT	Volume not sufficient for testing
		VR	Volume not sufficient for retest, if required
Q	Other quality concerns, not identified above (explain in COND_COMMENTS)		
COND_COMMENTS	Character	Explanation for Q FLAG (if needed)	
PARAMETER	Character	Analyte name	
METHOD	Character	Laboratory method used	
DATE PROCESSED Date that the analysis started	MMDDYY	Date that the analysis started	
HOLDING TIME Y/N Analysis performed within holding time	Y/N performed within holding time	Analysis performed within holding time	
MDL	Numeric	Lab method detection limit	
LRL	Numeric	Lab reporting limit	
DILUTION	Numeric	Dilution of sample (blank if no dilution)	
RESULT	Numeric	Concentration value	
REASON	Character	Reason for qualification in RESULT_QUAL	

Table 4. Continued.

Field	Format	Description
RESULT_QUAL	Character	Data qualifier
UNIT	Character	Unit of measurement for RESULT, MDL, and RL
QC_CODE	Character	Apply laboratory defined QC codes and describe in the comments field. Provide set of laboratory's code as part of the case narrative
COMMENT	Character	Explain situation that created QC code, or any unusual aspects

6.0 Sample QC

The laboratory will follow all QC/QA procedures for the analytical method being followed. The laboratory will also follow all laboratory in-house QA/QC procedures. QC activities include checking condition of samples upon arrival, storing sample appropriately, analyzing samples within the holding time, instrument calibration, and verifying all required data is recorded.

7.0 Sample Record Retention

The laboratory shall retain original records, including laboratory notebooks and the reference library, for a minimum of 5 years. After the stated time period, the laboratory shall follow its internal protocols for disposal.

8.0 References

- FGDC (Federal Geographic Data Committee). 2012. Coastal and Marine Ecological Classification Standard. June 2012. Marine and Coastal Spatial Data Subcommittee, Federal Geographic Data Committee. June 2012. FGDC-STD-018-2012. 343 pp.
- Kahn, L. 1988. Determination of Total Organic Carbon in Sediment (Lloyd Kahn Method). U.S. Environmental Protection Agency, Region II, Environmental Services Division, Monitoring Management Branch, Edison, New Jersey. 4 pp. Available at www.nj.gov/dep/srp/guidance/rs/lloydkahn.pdf.
- Rutecki, D. and E. Nestler. 2019. Massachusetts Estuaries Project, Benthic Monitoring Quality Assurance Project Plan (QAPP). Draft Revision 1. Prepared by Normandeau Associates, Inc., Falmouth, MA. March 2019. 105 pp.
- US EPA (U.S. Environmental Protection Agency). 2016. National Coastal Condition Assessment 2015: Laboratory Operations Manual. Version 2.1, May 2016. EPA-841-R-14-008. U.S. Environmental Protection Agency, Office of Water, Washington, DC. 214 pp.

IV. Laboratory Standard Operating Procedures for Underwater Still Images and/or Video

Digital still images and/or video collected as part of the soft-bottom infaunal grab samples or hard-bottom/riprap destructive samples will be used as a visual record of the bottom habitat at the sampling location. These images will only be analyzed as described below if specifically stated in the Embayment Specific Study Plan. Still images and video footage collected from stand-alone surveys will be analyzed as part of the survey following the procedures described below. Analysis of digital still images and video will require a trained analyst for habitat characterization and benthic macrofauna identification. For additional program details refer to the MEP Benthic Monitoring QAPP (Rutecki and Nestler 2019).

1.0 Digital Still Images

The following procedure will be used to analysis still images from macrofaunal sampling and stand-alone still image surveys.

1.1 Laboratory Equipment

- Still photograph captures (DVD or electronic file)
- A computer equipped with a photograph editing software

1.2 Procedure

Digital still image analysis begins with receipt of DVD or electronic copies of all images. The Chain of Custody form should be checked and filled out upon data acquisition. Digital sill analysis is typically accomplished using a square meter quadrat. However, in environments with poor visibility, a 0.5 x 0.5 meter quadrat is recommended to take the still photographs. Since visibility in shallow estuaries is typically poor, the 0.5 square meter quadrat and modified method may be required.

Digital still images collected for a still image survey will be analyzed using the following procedure. A total area of 1.0 x 1.0 meters will be analyzed in these images. If visibility was reduced and a 0.5 x 0.5 meter quadrat was used for the survey, combine the four photographs taken at each sample location into a single composite image covering the required 1.0 meter² area using a photograph editing software. Analyze each image for substrate type, habitat relief, sediment drape, and the relative abundance of macroalgae and macrofauna. Substrate types will be characterized for particle size following the CMECS sediment grain size descriptors (Table 5). Additional substrates that could be observed in digital images are presented in Table 6. Habitat relief, the difference in elevation between two surfaces, will be characterized as none, low, moderate, and high as described in Table 7. Drape, the visible layer of detrital material on the top of rock surface, will be characterized as absent, low, moderate, or heavy as described in Table 8. Relative abundance of macroalgae and macrofauna in each image will be determined by identifying organisms to the lowest practical taxon and using percent present per image. Percent present per image will be determined by using the photo editing software to place a grid template consisting of 100 squares over each image and counting the number of squares a species occurred in

for all species observed in the image. Each grid square represents 1% of the image. For digital still image surveys conducted along a transect, percent present for each species for the complete transect will be determined by averaging the percent present per image of all locations photographed along the transect. Relative abundance estimates of species in digital still images will be made based on the descriptions in Table 9. Evidence of fishing activities (e.g. trawl scars and lobster pots) and physical disturbances will be noted. The data from the video will initially be entered either on data sheets or directly into an Excel spreadsheet. Data entered on a datasheet will then be entered into an Excel spreadsheet. The spreadsheet will be delivered to the Project Manager.

Table 5. Sediment grain size descriptors (FGDC 2012).

Descriptor	Grain Size (millimeters)	Class Sizes (phi)
Clay	< 0.004	> 8
Silt	0.004 to < 0.0625	> 4 to 8
Mud	< 0.0625	> 4
Sand	0.0625 to < 2	4 to < -1
Very Fine Sand	0.0625 to < 0.125	4 to < 3
Fine Sand	0.125 to < 0.25	3 to < 2
Medium Sand	0.25 to < 0.5	2 to < 1
Coarse Sand	0.5 to < 1	1 to < 0
Very Coarse Sand	1 to < 2	0 to < -1
Gravel	2 to < 4,096	-1 to < -12
Granule	2 to < 4	-1 to < -2
Pebble	4 to < 64	-1 to < -6
Cobble	64 to < 256	-6 to < -8
Boulder	256 to < 4,096	-8 to < -12

Table 6. Additional substrates that could be observed in digital images.

Substrate	Description
Shell Hash	Surface substrate layers are dominated by loose shell accumulations with a median particle size of 2 mm to < 64 mm (granules and pebbles). Shells may be broken or whole (FGDC 2012).
Shell Reef Substrate	Substrate that is dominated by living or non-living cemented, conglomerated, or otherwise self-adhered shell reefs, with a median particle size of 4,096 millimeters or greater in any dimension. Live reef building fauna may or may not be present (FGDC 2012).
<i>Crepidula</i> Reef Substrate	Shell Reef that is primarily composed of conglomerated <i>Crepidula</i> shells (FGDC 2012).
Mussel Reef Substrate	Shell Reef that is primarily composed of self-adhered or conglomerated mussel shells (FGDC 2012).
Oyster Reef Substrate	Shell Reef that is primarily composed of cemented or conglomerated oyster shells (FGDC 2012).
Subcrop	Pieces of bedrock that have broken off but have not moved from its original location, or an occurrence of bedrock beneath a fairly flat-laying and widespread sediment deposit.
Talus	An accumulation of angular rock debris at the base of an outcrop that has occurred through periodic rockfall from the adjacent outcrop.

Table 7. Habitat relief descriptions.

Habitat Relief	Height (meters)
None	0
Low	0.1 to < 0.5
Moderate	0.5 to 2
High	> 2

Table 8. Drape categories and descriptions.

Drape	Description
Absent	Hard surface, encrusting, or fouling organisms are clearly visible
Low	A film of sediments covers less than 50% of the hard substrate or fouling organisms
Moderate	More than half of the hard substrate or organisms are covered or obliterated
High	Most of the hard substrate or encrusting/fouling organisms are covered and indistinguishable

Table 9. Relative abundance descriptions for digital still images.

Relative abundance	Percentage
Absent	The species was not observed in the still image
Rare	The species was observed in less than 1% of the still image or the transect average
Present	The species was observed in 1 to 25% of the still image or the transect average
Common	The species was observed in 26 to 50% of the still image or the transect average
Abundant	The species occurred in 51 to 100% of the still image or the transect average

2.0 Digital Video

The following procedure will be used to analysis video footage from macrofaunal sampling and stand-alone video surveys.

2.1 Laboratory Equipment

- High Definition digital video footage (DVD or electronic file)
- Still photograph captures (DVD or electronic file)
- Computer equipped with a monitor and video viewing software (for example VLC media player) and photograph editing software (for example Adobe Photoshop™)

2.2 Procedure

Video analysis begins with receipt of DVD or electronic copies of all video material. The Chain of Custody form should be checked and filled out upon data acquisition. The HD digital video footage will be reviewed for habitat characteristics and heterogeneity (i.e. substrate types, habitat relief, and sediment drape) and for biotic components. Substrate types will be characterized for particle size following the Coastal and Marine Ecological Classification Standard (CMECS) for sediment grain size (Table 5). Additional substrates that could be observed in the digital images are described in Table 6. Habitat relief, the

difference in elevation between two surfaces, for the macroscale (1 to 10 meters) features observed in the digital images will be characterized as none, low, moderate, and high (Table 7). Sediment drape is the visible layer of detrital material on the top of rock surfaces compose of fine-grain sediment, phytodetritus, zooplankton fecal pellets, tubes, and mucus. Drape will be characterized as absent, low, moderate, or heavy (Table 8). Biotic components will include the presence and general characterization of epibenthic invertebrates, finfish, and habitat. Organisms will be identified to the lowest practical taxonomic level using standard taxonomic keys for the geographic area. Relative abundance estimates of organisms in digital video footage will made based on the descriptions in Table 10. Evidence of fishing activities (e.g. trawl scars and lobster pots) and physical disturbances will be noted. Representative screen shots from the start, middle, and end of each video transect will be collected using the screenshot feature in a media player software (e.g. VLC media player). Additional still images may be extracted from the video if unique features or epibenthic organisms are observed. If still images are taken simultaneously with the digital video footage, the still images will be concurrently reviewed¹ for each transect and used to confirm benthic organism identification and estimates of relative abundance. The data from the video will initially be entered on data sheets and then into an Excel spreadsheet. The spreadsheet will be delivered to the Project Manager.

Table 10. Relative abundance descriptions for digital video footage.

Relative abundance	Description
Absent	The species was not observed in the video footage
Rare	The species was observed in less than 1% of the screens, i.e. length of the video monitor from top to bottom (representing, on average, 3 linear feet of substrate)
Present	The species was observed in 1 to 25% of the screens
Common	The species was observed in 25 to 50% of the screens
Abundant	The species was observed on more than 50% of the screens

3.0 Data Entry

All data generated by image analysts will be manually read from the instrument display (computer monitor) and entered directly into an electronic format (e.g., Excel spreadsheet), or entered into laboratory forms or data sheets, and then manually entered into an electronic format. All manually entered data will receive 100% verification or will be entered and checked using double data entry

¹ These still images are for verification purposes only and are not require to be analyzed following the procedures described in Section 1.0 above unless specifically requested in the Embayment Specific Study Plan.

Standardized codes and qualifiers help to ensure consistency over time in a benthic monitoring program. The underwater digital image codes are presented in Tables 11 and 12.

Table 11. Underwater digital image survey codes.

Field	Format	Description
SURVEY_NAME	Character	Name of sampling survey
VESSEL_NAME	Character	Name of the vessel used for the survey
CHIEF_SCIENTIST	Character	Name of the scientist in charge of the survey
STATION_ID	Character	Station identification code
STAT_ARRIV_LOCAL	Date	Station arrival date and time (local time)
BEG_LATITUDE	Numeric	Beginning latitude measured at each station (decimal degrees)
BEG_LONGITUDE	Numeric	Beginning longitude measured at each station (decimal degrees)
END_LATITUDE	Numeric	Ending latitude measured at each station (decimal degrees)
END_LONGITUDE	Numeric	Ending longitude measured at each station (decimal degrees)
NAVIGATION_CODE	Character	How station location was determined (e.g., LORAN-C, line of sight, survey map, etc.).
NAV_QUAL	Numeric	Estimated accuracy of navigation in meters.
DEPTH_TO_BOTTOM	Numeric	Depth to bottom in meters
COMMENTS	Character	Comments on survey detailing any exceptions from standard procedures

Table 12. Underwater digital image analysis data codes.

Field	Format	Description	
LAB NAME	Character	Name of lab	
STATION ID	Character	Station identification code	
DATE COLLECTED	MMDDYY	Date image was taken	
STAT_ARRIV_LOCAL	Date	Station arrival date and time (local time)	
IMAGE_DATE_TIME_BEG_LOCAL	Date	Video only: Time of the beginning of this video (local time)	
IMAGE_DATE_TIME_END_LOCAL	Date	Video only: Time of the end of this video (local time)	
USABLE_MINUTES	Numeric	Video only: Number of usable minutes between the <code>image_date_time_beg</code> and <code>image_date_time_end</code>	
DEPTH_BEG		Video only: Depth of water at <code>image_time_beg</code> (meters)	
DEPTH_END		Video only: Depth of water in at <code>image_time_end</code> (meters)	
DEPTH	Numeric	Still only: Depth of water in which the image was collected	
DATE ANALYSIS	MMDDYY	Date of analysis	
SUBS_CODE	Character	Codes describing the substrate observed in the digital image	
		Code	Definition
		b	Boulders
		c	Cobbles
		cpgp	Cobbles pavement, gravel pavement
		cp+ob	Cobble pavement and occasional boulders
		g	Gravel
		gp	Gravel pavement
		mm	Man-made rocks
		mx	Mix
		null	No primary substrate code given
		s	Sand
RELIEF_CODE	Character	Codes describing the habitat relief observed in the digital image	
		Code	Definition (See Table 12)
		n	None
		l	Low
		m	Moderate
		h	High

Table 12. Continued.

Field	Format	Description	
SED_DRAPE_CODE	Character	Codes describing the sediment drape observed in the digital image	
		Code	Definition (See Table 13)
		a	Absent
		l	Low
		h	High
SUSP_MATTER_CODE	Character	Codes describing the suspended matter observed in the digital image	
		Code	Definition
		h	High
		mh	Moderate to high
		vh	Very high
FAMILY	Character	Taxonomic family	
GENUS	Character	Taxonomic genus	
SPECIES	Character	Taxonomic species	
TAXA NAME	Character	Complete taxon name	
REL_ABUND	Character	Codes describing the suspended matter observed in the digital image	
		Code	Definition (See Tables 14 and 15)
		a	Absent
		r	Rare
		p	Present
		c	Common
		ab	Abundant
ANAL_COMMENTS	Character	General laboratory analysis comments	

4.0 Image Analysis QC

All appropriate high-definition video footage and still images will be analyzed. Video footage and still images will be examined for a range of substrate characteristics, sediment drape, and habitat relief; the occurrence of large identifiable taxa at each station; and evidence of fishing activities. Encrusting, cryptic, or very abundant taxa will not be counted from the video due to visual resolution and time constraints.

5.0 Sample Record Retention

The laboratory or contractor analyzing the images shall retain original records, images, video including laboratory notebooks and Excel spreadsheet files, for a minimum of 5 years. After the stated time period, the laboratory or contractor shall follow its internal protocols for file disposal.

6.0 References

- FGDC (Federal Geographic Data Committee). 2012. Coastal and Marine Ecological Classification Standard. June 2012. Marine and Coastal Spatial Data Subcommittee, Federal Geographic Data Committee. June 2012. FGDC-STD-018-2012. 343 pp.
- Rutecki, D. and E. Nestler. 2019. Massachusetts Estuaries Project, Benthic Monitoring Quality Assurance Project Plan (QAPP). Draft Revision 1. Prepared by Normandeau Associates, Inc., Falmouth, MA. March 2019. 105 pp.

V. Laboratory Standard Operating Procedures for Sediment Profile Imaging (SPI; Optional)

1.0 Laboratory Equipment

A computer equipped with a monitor and video reviewing software (for example VLC media player), photograph editing software (e.g. Adobe Photoshop™), and National Institutes of Health (NIH)ImageJ (open platform image analysis software) is required to analyze the SPI footage. Analysis of SPI data will require a trained analyst. For additional program details refer to the MEP Benthic Monitoring QAPP (Rutecki and Nestler 2019).

2.0 Procedure

The sediment profile images will be reviewed within seven business days of survey completion to provide a “quick look” analysis. Parameters that will be evaluated in the “quick look” analysis are:

- Sediment grain size- categorized following the CMECS sediment grain size descriptors (Table 5)
 - Sediment layering, thickness, and type
 - Surface and subsurface fauna and structures
 - Approximate prism penetration
 - Approximate surface relief
- Approximate apparent redox potential discontinuity (aRPD) depth - categorized following the CMECS depth modifiers (Table 13)
 - Other major, readily discernable patterns

Within one week of completion of the “quick look” review, the results will be communicated to the Project Manager via an email summary of the survey.

Each image file will be labeled with station and replicate data. The first analytical step is accomplished by visually examining the images and recording all observed features into a preformatted, standardized spreadsheet file. The parameters to be measured are summarized in Table 14 and discussed in more detail in Appendix D of Rutecki and Nestler (2019). Further details about these analyses can also be found in Rhoads and Germano (1986), Nilsson and Rosenberg (1997), Rosenberg et al. (2001), and Shumchenia and King (2010).

The videotapes also are analyzed visually, with all observed features also recorded into a preformatted, standardized spreadsheet. Photo editing software and NIH ImageJ are used to preprocess and analyze the three still images collected at each station. Computer analysis of each image is standardized by executing a series of macro commands. After visual and computer image analyses are completed, a standard set of parameters taken from both

analyses is combined and tabulated into an Excel spreadsheet. The SPI results, in the form of an Excel spreadsheet, will be delivered to the Project Manager.

3.0 Data Entry

All data generated by SPI analysts will be either electronically transferred from the instrument or manually read from the instrument display (video monitor) and entered directly into an electronic format (e.g., Excel spreadsheet). Standardized codes and qualifiers help to ensure consistency over time in a benthic monitoring program. Tables 15 and 16 show the parameters codes for the SPI survey and analysis. Prior to the release of any data from SPI analysis, the data will be reviewed and approved by a senior analyst.

Table 13. aRPD Depth Modifier (FGDC 2012).

aRPD Depth Values	aRPD Depth (centimeters)
Zero	0.0
Diffusional	> 0.0 to 1.0
Shallow	> 1.0 to 2.0
Moderate	> 2.0 to 3.5
Deep	> 3.5 to 5.0
Very Deep	> 5

Table 14. Parameters Measured from Sediment Profile Images

Parameter	Units	Method ¹	Description
Sediment Grain Size	phi (Φ)	V	An estimate of sediment types present. Determine by comparison of image to images of known grain size.
Prism Penetration	cm	CA	A geotechnical estimate of sediment compaction. Average of maximum and minimum distance from sediment surface to bottom of prism window
Sediment Surface Relief	cm	CA	An estimate of small-scale bed roughness. Maximum depth of penetration minus minimum.
Apparent Reduction-oxidation Potential Discontinuity Depth (from color change in sediment)	cm	CA	Estimate of depth to which sediments are oxidized. Area of aerobic sediment divided by width of digitized image.
Surface Features			
Pelletal Layer	—	V	Note if present
Bacterial Mats	—	V	If present, note color
Epifauna	—	V	If present, note and identify
Submerged aquatic vegetation	—	V	Note if present
Tubes			
• Type	—	V	Identify as amphipod or polychaete
• Density	Number	V	Estimate number (none, few, some, many)
Subsurface Features			
Methane/Nitrogen Gas Voids	Number	V	Count
Infauna			
• Visible Infauna	Number	V	Count, identify
• Burrow Structures Feeding (Oxic) Voids	—	V	Count
• Successional Stage	Number	V	Count
• Successional Stage	—	V	Identify
Organism-Sediment Index (OSI)	—	CA	Derived from aRPD, Successional Stage, and Voids (Rhoads and Germano 1982, 1986)

¹V: Visual measurement or estimate
CA: Computer analysis

Table 15. Sediment profile imaging survey data codes.

Field	Format	Description
SURVEY_NAME	Character	Name of sampling survey
VESSEL_NAME	Character	Name of the vessel used for the survey
CHIEF_SCIENTIST	Character	Name of the scientist in charge of the survey
STAT_ID	Character	Station
STAT_ARRIV_LOCAL	Date	Station arrival date and time (local time)
BEG_LATITUDE	Numeric	Beginning latitude measured at each station (decimal degrees)
BEG_LONGITUDE	Numeric	Beginning longitude measured at each station (decimal degrees)
END_LATITUDE	Numeric	Ending latitude measured at each station (decimal degrees)
END_LONGITUDE	Numeric	Ending longitude measured at each station (decimal degrees)
NAVIGATION_CODE	Character	How station location was determined (e.g., LORAN-C, line of sight, survey map, etc.).
NAV_QUAL	Numeric	Estimated accuracy of navigation in meters.
DEPTH_TO_BOTTOM	Numeric	Depth to bottom in meters
COMMENTS	Character	Comments on survey detailing any exceptions from standard procedures

Table 16. Sediment profile imaging analysis parameter codes.

Field	Format	Unit	Description
LAB_NAME	Character		Name of lab
SITE_ID	Character		Site identification code
DATE_COLLECTED	MMDDYY		Date image was taken
DATE_ANALYSIS	MMDDYY		Date image was analyzed
ANOXIC_VOID_NUM	Numeric		Number of water-filled spaces in sediment that appear to be abandoned feeding voids
AVG_PEN	Numeric	cm	Average penetration
AVG_RPD	Numeric	cm	Average depth of the apparent color redox potential discontinuity layer
BURR_NO	Numeric		Number of burrows
GAS_VOID_NUM	Numeric		Number of gas filled spaces in sediment resulting from methanogenesis
GRN_SZ	Numeric		Sediment grain size
OSI	Numeric		Organism-Sediment Index
OXIC_VOID_NUM	Numeric		Number of active, water-filled spaces in sediment resulting from sub-surface feeding activity of infauna
PEN_MAX	Numeric	cm	Maximum penetration depth of camera
PEN_MIN	Numeric	cm	Minimum penetration depth of camera
RPD_MAX	Numeric	cm	Maximum depth of the apparent color redox potential discontinuity layer
SR	Numeric	cm	Surface relief across the 15 cm width of the face plate. Calculated as (PEN_MAX – PEN_MIN)
SUB_FAUNA_WORMS	Numeric		Infaunal worms counted
SUCC_STG	Numeric		Estimated infaunal successional stage
SUR_FEATURES	Numeric		Features on the sediment surface
TUBE_AMPH	Numeric		Amphipod tube
TUBE_POLY	Numeric		Polychaete tube

4.0 Analysis QC

The QC objectives for SPI analysis are that (1) at least three images from each station be analyzed, (2) all parameters defined in this SOP and in the Benthic Monitoring QAPP (Rutecki and Nestler 2019) be analyzed for all images, and (3) that analytical systems used enable repeatable measurements and determinations to be made.

The comparability of the SPI analyses will be ensured by using the same analyst throughout the project whenever possible. Slight variation in the manner in which the analyst examines the slide may occur. This may result in a slight variation of image areas analyzed within and between slides. To control for analyst error, 10% of all slides will be reanalyzed and the results compared to previous results. If any discrepancies with the original analysis are found then all images will be checked and reanalyzed.

5.0 Sample Record Retention

The SPI contractor shall retain original records, images, video including laboratory notebooks and Excel spreadsheet files, for a minimum of 5 years. After the stated time period, the laboratory shall follow its internal protocols for disposal.

6.0 References

- FGDC (Federal Geographic Data Committee). 2012. Coastal and Marine Ecological Classification Standard. June 2012. Marine and Coastal Spatial Data Subcommittee, Federal Geographic Data Committee. June 2012. FGDC-STD-018-2012. 343 pp.
- Nilsson, H.C., and R. Rosenberg. 1997. Benthic habitat quality assessment of an oxygen stressed fjord by surface and sediment profile images. *Journal of Marine Systems* 11: 249–264.
- Rhoads, D.C. and J.D. Germano. 1986. Interpreting long-term changes in benthic community structure: a new protocol. *Hydrobiologia* 142: 291–308.
- Rosenberg, R., H.C. Nilsson, and R.J. Diaz. 2001. Response of benthic fauna and changing sediment redox profiles over a hypoxic gradient. *Estuarine Coastal and Shelf Science* 53: 343–350.
- Rutecki, D. and E. Nestler. 2019. Massachusetts Estuaries Project, Benthic Monitoring Quality Assurance Project Plan (QAPP). Draft Revision 1. Prepared by Normandeau Associates, Inc., Falmouth, MA. March 2019. 105 pp.
- Shumchenia, E.J., and J.W. King. 2010. Evaluation of sediment profile imagery as a tool for assessing water quality in Greenwich Bay, Rhode Island, USA. *Ecological Indicators* 10: 818–825.