Massachusetts Estuaries Project
Marine Benthic Monitoring
Quality Assurance Project Plan

Prepared for:
Watershed Planning Program
Division of Watershed Management, Bureau of Water Resources
Massachusetts Department of Environmental Protection

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Cover Photo
Photos by Normandeau Associates, Inc. Images of benthic habitat from Pleasant Bay 2020 survey, including eelgrass, a horseshoe crab, and quahog shell rubble. *Massachusetts Estuaries Project-Benthic Monitoring Report: Pleasant Bay 2021*. MassDEP. June 2021.

A. PROJECT MANAGEMENT

A1. TITLE AND APPROVALS

Review and Approvals

Richard O. Carey

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July 17, 2023

Richard O. Carey, Ph.D. Date
Director, Watershed Planning Program
Massachusetts Department of Environmental Protection

Matthew Reardon

June 29, 2023

Matthew Reardon Date
Total Maximum Daily Load Section Chief
Watershed Planning Program
Massachusetts Department of Environmental Protection

Suzanne M. Flint

June 29, 2023

Suzanne Flint Date
Quality Assurance Analyst
Watershed Planning Program
Massachusetts Department of Environmental Protection

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A3. DISTRIBUTION LIST

Copies of this QAPP, and any subsequent revisions, will be distributed by the Watershed Planning Program, Massachusetts Department of Environmental Protection (MassDEP), after approvals have been obtained. The following groups will be made aware of this QAPP:

- MassDEP QA Managers (Bureau of Water Resources)
- MassDEP, Division of Municipal Services
- Massachusetts Office of Coastal Zone Management (Todd Callaghan)
- Executive Office of Energy and Environment (EEA) (Joe Costa)
- MassBays (Pam Dibona)
- US Environmental Protection Agency (US EPA) – Region 1 (relevant staff)
- Cape Cod Commission (relevant staff)
- Buzzards Bay Coalition (Rachel Jakuba)
- Massachusetts Maritime Academy (William Hubbard)

Electronic copies of this QAPP are available on the MassDEP website: https://www.mass.gov/guides/the-massachusetts-estuaries-project-and-reports.

A4. PROJECT AND TASK ORGANIZATION

A4.1 QAPP Introduction

This Quality Assurance Project Plan (QAPP) presents the organization, objectives, functional activities, and specific quality assurance (QA) and quality control (QC) activities associated with the Marine Benthic Monitoring that will be conducted through the Massachusetts Estuaries Project (MEP). This document also describes the specific protocols that will be followed for sampling, sample handling and storage, chain of custody, laboratory and field analyses, data review and validation, document management, data management, and data usability assessment. All applicable federal, state, and local laws and regulations are to be followed when conducting activities described in this Quality Assurance Project Plan.

This QAPP was prepared in accordance with US EPA guidance documents U.S. EPA QA/R-5 (2001, reissued 2006) and U.S. EPA QA/G-5 (2002). Separate standard operating procedures (SOPs) will supplement this QAPP. The SOPs will provide the additional operational details required to conduct benthic sampling, and will describe staff responsibilities, schedule details, and specific equipment.

A4.2 Project Organization

The Benthic Monitoring tasks will be accomplished through the coordinated efforts of personnel from MassDEP, Cape Cod Commission, Buzzards Bay Coalition and other nonprofit

organizations, Massachusetts Maritime Academy and other educational institutions, coastal communities, consulting firms, and volunteer programs.

MassDEP

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

- Dr. Richard Carey, Director, Watershed Planning Program (WPP).
- Mr. Matthew Reardon, Total Maximum Daily Load (TMDL) Section Chief. Mr. Reardon has primary oversight of the program.

Town(s) or Teaming Partner

The town(s) or teaming partner ("Town") undertaking the embayment work described in this QAPP will designate a person, committee, or contracted entity to coordinate with MassDEP and oversee specific tasks detailed in this QAPP. The designated person, committee, or entity will be the "Project Manager" and fulfill those responsibilities.

Specific project roles for project participants are summarized in Table 1.

Table 1. Program roles and responsibilities related to monitoring and data use for Benthic Monitoring Program.

Personnel, Title and/or Primary Role	Responsibilities
Richard Carey, Director, Watershed Planning Program (WPP)	Overall management of administrative and technical work conducted by WPP staff.
Matthew Reardon, TMDL Section Chief, WPP	Oversee development and implementation of nutrient Total Maximum Daily Loads (TMDLs) for State waters. Primary MassDEP staff member responsible for overseeing benthic monitoring work described in this QAPP.
Andrew Osei, Wastewater Management - Cape and Islands	Representing MassDEP to serve as an interface between other MassDEP, EPA and Town officials.
Suzanne Flint, Quality Assurance Analyst, WPP	Overall technical oversight of project data QA/QC and management.
Project Manager	Primary person or entity responsible for overseeing benthic monitoring work described in this QAPP. Serves as an interface between the Town and MassDEP officials.
QA Manager	Primary person responsible for project QA/QC procedures.
Chief Scientist	Primary person responsible for overseeing the quality of work and data collected in the field.
Survey Crew (Town staff, seasonal employees, consultants, and volunteers as needed)	Under the direction of the Chief Scientist, the survey crew follows the MEP Field SOP to collect benthic samples and data.
Laboratories under contract	Overall lab management and technical oversight regarding the performance of benthic fauna taxonomic identification and sediment analyses, and submittal of validated data to the Project Manager in compliance with contractual arrangements.

A5. BACKGROUND

A5.1 Background

Coastal embayments throughout the Commonwealth of Massachusetts are becoming nutrient enriched primarily due to changes in watershed land-use associated with increasing population within the coastal zone over the past half century. Many of Massachusetts' embayments have nitrogen levels that are approaching or are currently over the assimilative capacity, which causes declines in their ecological health resulting in the loss of fisheries habitat, eelgrass beds, and a general disruption of benthic communities and the food chain they support. High levels of nitrogen loading from surrounding watersheds can cause aesthetic degradation (e.g., large algae blooms) and inhibit even recreational uses of coastal waters (Valiela et al. 1997, Valiela and Bowen 2002, Carmichael et al. 2004, Williamson et al. 2017, Howes et al. 2017).

The Massachusetts Department of Environmental Protection (MassDEP) in conjunction with its technical partners, UMass-Dartmouth School of Marine Science and Technology, the Cape Cod Commission and the U.S. Geological Survey, established the Massachusetts Estuaries Project (MEP) in 2001 to monitor and protect estuarine ecosystems in southeastern Massachusetts embayments. The goals and approach of the MEP are described by Howes et al. (2003) in a report entitled, *Site-Specific Nitrogen Thresholds for Southeastern Massachusetts Embayments: Critical Indicators*. The stated goal of the MEP was to assess the current condition of 89 embayments in southeastern Massachusetts 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 (Howes et al. 2003). The essential component of the MEP was the development of site-specific critical thresholds for the coastal embayments within the study region based on specific basin configuration, source water quality, and watershed spatial features for each embayment (Howes et al. 2003). These thresholds were developed using a process that relied on scientifically credible principles and approaches, following the established regulatory framework governing surface water quality management in the Commonwealth of Massachusetts. Water quality indicators including dissolved oxygen, ecological diversity, and the presence of certain animal and plant species were selected based on being either 1) an essential component of all estuarine habitat health criteria, 2) of proven utility in southeastern Massachusetts embayments, or 3) supported by the Linked Watershed-Embayment Management Model (Howes et al. 2003).

A Quality Assurance Project Plan (QAPP) for the MEP was developed in 2003 (Howes and Samimy 2003) and defined specific project tasks required to fulfill all the data needs and goals of the MEP and the use of the Linked Watershed-Embayment Management Model Approach. The Linked Watershed-Embayment Management Model Approach is a quantitative tool that links watershed inputs with embayment circulation and nitrogen characteristics to assess specific areas within embayments (Howes et al. 2001). The QAPP provided site-specific details for Embayments 1 through 34, field protocols, data sheets, and chain of custody forms. The QAPP was revised 2004 (Embayments 35 through 49) and 2005 (Embayments 50 through 64; UMass Dartmouth 2004, Howes and Samimy 2005).

Approximately 70 estuaries in southeastern Massachusetts have been assessed since the start of the MEP. The technical reports for these estuaries document baseline water quality and identify the actions required to restore nutrient-impaired waters. MEP has provided technical guidance in support of policies on nitrogen loading to embayments, wastewater management decisions, and establishment of nitrogen Total Maximum Daily Loads (TMDLs). A TMDL represents the maximum amount of a pollutant that a waterbody can assimilate and still meet the Massachusetts Surface Water Quality Standards for protection of public health and aquatic life (314 CMR 4.00). TMDLs for Total Nitrogen have been established for over 30 estuaries. Many communities have begun the process of integrated water resources management planning or preparing Comprehensive Wastewater Management Plans (CWMPs). With implementation of the TMDLs and community measures, MassDEP identified a need to review and update the benthic infauna survey procedures that were created in 2003. The goal of this document is to

outline the specific organization, objectives, functional activities, and QA and QC activities to be used by MassDEP and parties outside MassDEP to collect benthic data that will be of sufficient quality to be used in the management decisions for southeastern Massachusetts embayments.

A5.2 Regulatory Overview

The regulatory basis for the MEP is provided by the Massachusetts Surface Water Quality Standards (SWQS; 314 CMR 4.00), which implement provisions of the federal Clean Water Act[1]. The SWQS designate the most sensitive uses of Massachusetts surface waters and provide criteria for evaluating water quality to support those uses. The current SWQS, as promulgated in 2021, set forth classifications for coastal and marine waters. Criteria that support the uses for these classifications are both numeric and narrative. The three classes for coastal and marine waters are SA, SB, and SC and are defined as follows at 314 CMR 4.05(4): *Coastal and Marine Classes*:

Class SA - "*These waters are designated as an excellent habitat for fish, other aquatic life and wildlife, including for their reproduction, migration, growth and other critical functions, and for primary and secondary contact recreation. In certain waters, excellent habitat for fish, other aquatic life and wildlife may include, but is not limited to, seagrass. Where designated for shellfishing in 314 CMR 4.06(6)(b), these waters shall be suitable for shellfish harvesting without depuration (Approved and Conditionally Approved Shellfish Areas). These waters shall have excellent aesthetic value.*"

Class SB - "*These waters are designated as a habitat for fish, other aquatic life and wildlife, including for their reproduction, migration, growth and other critical functions, and for primary and secondary contact recreation. In certain waters, habitat for fish, other aquatic life and wildlife may include, but is not limited to, seagrass. Where designated for shellfishing in 314 CMR 4.06(6)(b), these waters shall be suitable for shellfish harvesting with depuration (Restricted and Conditionally Restricted Shellfish Areas). These waters shall have consistently good aesthetic value.*"

Class SC - "*These Coastal and Marine Waters are designated as a habitat for fish, other aquatic life and wildlife, including for their reproduction, migration, growth and other critical functions, and for secondary contact recreation. They shall also be suitable for certain industrial cooling and process uses. These waters shall have good aesthetic value.*"

Specific criteria for dissolved oxygen, temperature, pH, bacteria, solids, color and turbidity, oil and grease, and taste and odor have been designated for each classification (see 314 CMR 4.05(4)(a), (b), and (c)). Additionally, the regulation applies minimum criteria to all surface

[1] DISCLAIMER: The descriptions of the current SWQS regulation included in this document are for informational purposes, only. The actual SWQS regulation shall control in the event of any discrepancy with the description provided. As a result, no person in any administrative or judicial proceeding shall rely upon the content of this document to create any rights, duties, obligations or defenses, implied or otherwise, enforceable at law or in equity.

waters for toxic pollutants at 314 CMR 4.06(6)(d): *Table 29: Generally Applicable Criteria*, as well as aesthetics, bottom pollutants or alterations, nutrients and radioactivity at 314 CMR 4.05(5):

Aesthetics - *"All surface waters shall be free from pollutants in concentrations or combinations that settle to form objectionable deposits; float as debris, scum or other matter to form nuisances; produce objectionable odor, color, taste or turbidity; or produce undesirable or nuisance species of aquatic life."*

Bottom Pollutants or Alterations - "*All surface waters shall be free from pollutants in concentrations or combinations or from alterations that adversely affect the physical or chemical nature of the bottom, interfere with the propagation of fish or shellfish, or adversely affect populations of non-mobile or sessile benthic organisms.*"

Nutrients - "*Unless naturally occurring, all surface waters shall be free from nutrients in concentrations that would cause or contribute to impairment of existing or designated uses and shall not exceed the site-specific criteria developed in a TMDL or as otherwise established by the Department pursuant to 314 CMR 4.00 including, but not limited to, those established in 314 CMR 4.06(6)(c): Table 28: Site-specific Criteria. Any existing point source discharge containing nutrients in concentrations that would cause or contribute to cultural eutrophication, including the excessive growth of aquatic plants or algae, in any surface water shall be provided with the most appropriate treatment as determined by the Department, including, where necessary, highest and best practical treatment (HBPT) for POTWs and BAT for non-POTWs, to remove such nutrients to ensure protection of existing and designated uses. Human activities that result in the nonpoint source discharge of nutrients to any surface water may be required to be provided with cost effective and reasonable best management practices for nonpoint source control.*"

The nutrient, dissolved oxygen, and bacteria criteria in the SWQS relate most directly to the MEP; however, the bottom pollutant or alteration and aesthetic narrative criteria and potentially other criteria listed at 314 CMR 4.06(6)(d): *Generally Applicable Criteria*, should also be considered. Under the SWQS, almost all the habitat health requirements are set forth under the "nutrient" narrative criteria at 314 CMR 4.05(5)(c), which refer to both site-specific criteria and control of cultural eutrophication. This provides a mechanism for linking the current classification system in the SWQS with more detailed habitat health criteria (habitat indicators; Howes et al. 2003).

MassDEP uses the evaluations conducted by the MEP to determine nitrogen-impaired embayments, identify the sources of nitrogen pollution, and develop TMDLs for each assessed embayment to protect and restore water quality. Development of TMDLs is required pursuant to Section 303(d) of the federal Clean Water Act for waters that do not meet the Massachusetts Surface Water Quality Standards. TMDLs must identify point and nonpoint sources of the pollutant of concern, the allowable load to meet water quality standards, and then allocate that load to all sources taking into consideration a margin of safety, seasonal variations, and several other factors. Each TMDL must also contain an outline of an implementation plan. MassDEP recognizes that restoring polluted waters is a long-term process and there are likely to be

multiple ways to achieve the required goals, some of which are more cost effective than others. Therefore, it is important for each Town to further evaluate potential options suitable to their community. MassDEP will work with the Town and will likely recommend that specific activities and timelines be further evaluated and developed by the Town through the Comprehensive Wastewater Management Planning process. Once a plan is developed and fully implemented, the embayment will need to be routinely re-evaluated to confirm that water quality has improved and that the required goals have been and are continuing to be met.

A5.3 Scientific Perspective

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 dissolved oxygen) to highly stressed (high organic matter, low dissolved oxygen). Communities in benthic assemblages respond to a variety of stressors in different ways allowing the type of stress that has affected 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 2015a). This approach has been accepted in the regulatory community in relation to pollution (e.g., oil, metals, and nutrients) and effects on estuarine and marine habitats for decades (Pearson and Rosenberg 1978, Rhoads and Germano 1986, Weisberg et al. 1997, US EPA 2015b). Since 2003, the MEP has followed the approach used in the pollution-related efforts where pollution tolerance of individual species allows their use as indicators (Howes et al. 2003, Howes and Samimy 2003). MEP will continue to use this approach as outlined in this document and the related standard operating procedures. Nutrient related tolerance (e.g., organic matter loading) is used as the primary factor (Howes et al. 2003).

Increased nitrogen concentrations in coastal embayments from point and nonpoint sources affect organic matter loading, increasing organic matter content of the sediments and resulting in increased sulfide concentrations. This results in a decrease in the level of sediment oxidation, which can reach the surface at the highest levels of organic input. Benthic infaunal and epifaunal communities associated with increasing nitrogen loading shift in response to the resultant increase in organic matter deposition to the sediments. Benthic infaunal species from sediment samples are identified and categorized to their tolerance or sensitivity to nutrient related stresses (Howes et al. 2003).

A5.3.1 *Objectives and Scope*

The objectives of the MEP Marine Benthic Monitoring Program are to (1) reassess ecological health for embayments that have been previously assessed under MEP, (2) evaluate the ecological health of southeastern Massachusetts embayments that have not been assessed, (3) assess how management actions have affected the embayment, and (4) determine if long-term changes are occurring in southeastern Massachusetts embayments that may indicate stress from eutrophication or other factors including invasive species and climate change.

The principal objective of the benthic surveys is to determine the chemical and biological health of an embayment and any sub-embayments in a system by assessing the sediment and benthic macroinvertebrate communities. This includes the documentation of improvement or continued impairment of embayments where TMDLs have been implemented. These objectives are addressed by five major tasks. Task 1 focuses on sampling design and planning. Task 2 focuses on the sampling activities that will take place in the embayment to be assessed. Task 3 includes the analysis of the collected samples and benthic images. Task 4 presents the chemistry and faunal data and the overall ecological health of the embayment in a report. Task 5 uploads the data to a database. The report and notification of data submission to MassDEP (Task 6) will begin a review and evaluation process that will lead to future coordination with MassDEP.

Embayment Specific Study Plan (Task 1) includes the development of a plan to conduct benthic macrofaunal surveys within a specific embayment. The plan would outline the approach selected for either previously assessed or unassessed embayments as described in the guidance document *Benthic Macrofaunal Monitoring Guidance to Support TMDLs and Habitat Condition Assessments*. For previously assessed embayments the plan would include (1) a review of the MEP technical report conclusions from the previous survey(s), current TMDL implementation and/or community measures undertaken, and embayment data currently available including a description of the habitat types in embayment with maps; (2) selection of a tiered sampling plan; (3) a map and the coordinates (i.e. decimal degrees latitude and longitude) of the re-established benthic infaunal stations previously sampled and any additional locations of interest; and (4) a survey plan that provides the operational details required to conduct each survey including participating staff, schedule, and specific equipment. For embayments not previously assessed, the plan would include (1) a description of the embayment; (2) review of existing embayment data; (3) development of an embayment-specific sampling design using a Generalized Random Tessellation Stratified (GRTS) approach to determine the location and number of sites to be sampled (See Section B1); (4) a map of the benthic sampling locations; and (5) a survey plan that provides the operational details required to conduct the baseline approach including participating staff, schedule, and specific equipment.

Benthic Monitoring Surveys (Task 2) include, soft-bottom infaunal surveys using traditional grab sampling methods and underwater digital images using high-definition (HD) digital cameras to characterize the physical, chemical, and biological status of habitats at the benthic stations defined in the Embayment Specific Study Plan. Work will include a pre-survey access review or site evaluation and plan for site substitution as needed. Water quality measurements and digital images will be taken with macrofaunal sampling to provide data on factors influencing the benthic assemblages. Optional benthic monitoring methods that may be included in the Embayment Specific Study Plan are Sediment Profile Imaging (SPI; Section B2.2.6) and hard bottom/riprap surveys (Section B2.2.7).

Analysis of Benthic Surveys (Task 3) include the determination of the sediment grain size, total organic carbon (TOC), water quality, and/or benthic macrofauna community

structure. Depending on the approach and optional methods selected, the benthic community structure determination under this task could include (1) the identification and enumeration of the benthic fauna recovered from sediment grab and/or hard-bottom destructive samples collected under Task 2, (2) the analysis of water quality measurements, (3) the analysis of digital images or video, and (4) the analysis of SPI images. Results will be evaluated statistically to characterize benthic community structure and to assess embayment ecological health. A reference collection of all macrofaunal taxa (identified and unidentified specimens) will be stored, maintained, and compiled throughout the project.

Synthesis Report (Task 4) includes the preparation of a summary report in which data developed under Tasks 2 and 3 will be presented. The report will evaluate current sediment condition, the status of benthic community, and the ecological health of the embayment. The report will be provided to the Town and the MassDEP MEP Program Manager. Accompanying electronic files for the Synthesis Report will include all electronic data files such as water quality measurements, benthic macrofauna data files, SPI data files, and underwater digital images.

Upload Data to the Selected Database (Task 5) includes uploading the data generated during the assessment process (Tasks 2 and 3) to the database selected by MassDEP (e.g., US EPA Water Quality Exchange Web [WQX Web]) so that the data are available to MassDEP and the public.

Future Coordination with MassDEP (Task 6) includes submitting the final Synthesis Report to MassDEP and notifying MassDEP that the data has been uploaded to the database. This will initiate a review and evaluation process by MassDEP.

A6. PROJECT/TASK DESCRIPTION

A6.1 Embayment Benthic Surveys

MEP surveys provide benthic samples and other data that can document the current conditions of benthic communities in an embayment and long-term trends in sediment quality and benthic assemblages following changes in coastal community development, implementation of TMDLs and reduction in nutrient loading to a system, or other changes including major storm events and shifts in species distribution from invasive species or climate change.

Benthic monitoring surveys (Task 2) will be conducted in August – October to be consistent with previous MEP benthic surveys, the US EPA National Coastal Condition Assessment (NCCA), and other studies in Massachusetts coastal waters. The number and locations of soft-sediment grab samples, digital still images or video, SPI (optional), and/or hard bottom/riprap destructive samples (optional) are embayment specific and will be described in the Embayment Specific Study Plan (Task 1). Soft-bottom benthic grab samples will provide local detail of the

sediment and infaunal communities inhabiting the embayment. HD digital images taken at sampling stations with a HD digital video camera will provide a visual record of the benthic communities and can be reviewed at a later date if a more detailed analysis is required. An SPI survey provides an area-wide, qualitative/semi-quantitative assessment of sediment quality and benthic community status that can be integrated with the results of the quantitative surveys to determine sedimentary conditions in the embayment. Sediment profile imagery allows a faster evaluation of the benthos compared to traditional faunal analyses; this survey will allow a rapid determination of benthic conditions in large embayments or coastwide assessments including the sediment depth of the apparent Redox Potential Discontinuity (aRPD). SPI can be used to determine locations where more in-depth analysis, such as benthic grab samples, may need to be considered. Hard-bottom/riprap destructive samples provide local detail of epifaunal communities inhabiting hard structures in the embayment. Following faunal identification and enumeration, data from the sampling locations will be analyzed for benthic macrofaunal community parameters (Task 3).

The sampling approach selected by the town(s) or teaming partner in consultation with MassDEP will depend on multiple factors, including: 1) whether the embayment has been previously assessed or is unassessed, 2) in previously assessed embayments, the embayment habitat health documented in the last assessment, and 3) the number of years between assessments. The recommended sampling approaches are presented in Tables 2 and 3. The sampling approaches are described in the guidance document *Benthic Macrofaunal Monitoring Guidance to Support TMDLs and Habitat Condition Assessments.* The conceptual framework for this benthic monitoring envisions the initial step of complete reassessment of a previously assessed embayment (when data are older than 5 years) or baseline assessment of an unassessed embayment followed by partially reassessment of an embayment every three years (step 2). The embayment will then be reassessed more completely every sixth year based on the embayment health documented in the prior assessment (step 3), re-starting the assessment cycle (Figure 1). The sampling approach selected will be identified in the Embayment Specific Study Plan (Task1). The station locations and number of stations to be sampled will be embayment-specific based on the size and complexity of the estuary being assessed, and the approach selected. Stations in previously assessed embayments will be determined in consultation with MassDEP and re-established using GIS software to allow for comparison between the initial and future assessments. The location and number of stations to be sampled for unassessed embayments will be determined using a Generalized Random Tessellation Stratified (GRTS) survey design consistent with the NCCA approach.

An access review of sample locations in previously assessed embayments or a site evaluation for unassessed embayments will be performed prior to the start of field work to identify issues with access to small or narrow sub-embayments. An access review provides an opportunity to identify any problems with access before sampling and allows for a discussion with MassDEP, the Town, or teaming partner to determine the importance of the stations in the area and possible alternative locations. A site evaluation will ensure sampling locations and alternative locations meet the target population identified in the selected GRTS survey design. The

evaluation will also provide initial verification of site suitability and further align the MEP benthic monitoring protocols with the NCCA and Massachusetts Coastal Condition Assessment programs. A desktop evaluation is recommended for both the access review and site evaluation to help minimize costs.

Details of field collection, sample handling, and laboratory methods to be used in the benthic monitoring surveys are provided in Sections B-2, B-3, and B-4, respectively, as well as in the Field and Laboratory SOPs in Appendices A and B.

Table 2. Summary of recommended sampling approach with analysis methods for previously assessed embayments in the Massachusetts Estuaries Program.

Approach	Tier 1	Tier 2
Sampling Frequency	Every 3 Years	Every 6 Years
Sampling Locations	MassDEP to determine based on prior assessments Priority given to stations with most impaired benthic habitats (up to 50% of previously sampled benthic infaunal stations)	MassDEP to determine based on prior assessments (All previously sampled benthic infaunal stations)
Sampling Method[1]		
Sediment Grain Size	x	x
Total Organic Carbon (TOC)	x	x
Benthic Infaunal Sampling - with a van Veen grab	x (Analyze total sample to the species level)	x (Analyze total sample to the species level)
Digital Images of the Substrate Surface - at least 2 still images per station taken prior to grab sampling	x	x
Water Quality - measurements for water temperature, dissolved oxygen, pH, and salinity taken prior to grab sampling	x	x
Analysis		
Community Parameters[2]	x	x
Multivariate Analyses[3]	x	x
US-M-AMBI	x	x

[1]Optional sampling methods that may be used for MEP benthic monitoring in addition to the methods listed above include a stand-alone digital video benthic survey, Sediment Profile Imagery (SPI), and hard-bottom/ riprap destructive samples.

[2]Community Parameters = abundance, H' diversity, J' evenness, Margalef, Simpson, and AvTD.

[3]Multivariate analyses = Bray-Curtis similarity hierarchical agglomerative clustering (cluster analysis) and non-metric multidimensional scaling (nMDS).

Table 3. Summary of recommended sampling approach with analysis methods for southern Massachusetts embayments unassessed by the Massachusetts Estuaries Program.

Approach	Baseline
Sampling Locations	Stations determined using a Generalized Random Tessellation Stratified survey design (All stations). MassDEP to approve all sampling locations
Sampling Method[1]	
Sediment Grain Size	x
Total Organic Carbon (TOC)	x
Benthic Infaunal Sampling - with a van Veen grab	x (Collect samples at all stations, analyze total sample to the species level)
Digital Images of the Substrate Surface - at least 2 still images per station taken prior to grab sampling	x
Water Quality - measurements for water temperature, dissolved oxygen, pH, and salinity taken prior to grab sampling	x
Analysis	
Community Parameters[2]	x
Multivariate Analyses[3]	x
US-M-AMBI	x

[1]Optional sampling methods that may be used for MEP benthic monitoring in addition to the methods listed above include a stand-alone digital video benthic survey, Sediment Profile Imagery (SPI), and hard-bottom/ riprap destructive samples.

[2]Community Parameters = abundance, H' diversity, J' evenness, Margalef, Simpson, and AvTD.

[3]Multivariate analyses = Bray-Curtis similarity hierarchical agglomerative clustering (cluster analysis) and non-metric multidimensional scaling (nMDS).

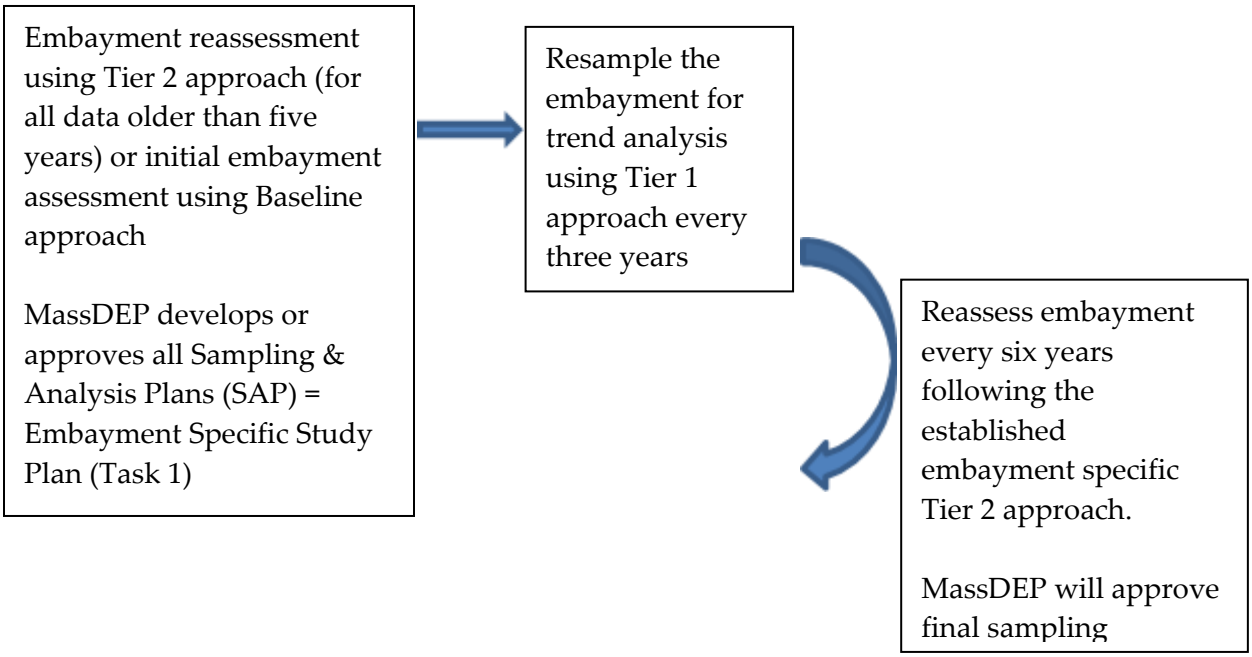

Figure 1. Conceptual framework for the Massachusetts Estuaries Project Marine Benthic Monitoring Program.

A6.2 Schedule of Deliverables and Activities

Benthic monitoring activities conducted for the MEP assessment will begin when an Embayment Specific Study Plan is submitted to MassDEP in February of a survey year and span the period through May of the following year when the final synthesis report is due. Deliverables include the embayment study plan, survey summary emails, reference collection, and synthesis report with data submission (prepared under Task 4). Schedule for these deliverables and activities are outlined in Table 4 below.

Table 4. Schedule of Benthic Monitoring Deliverables.

Task	Deliverable	Due Dates
Task 1. Embayment Specific Study Plan	Embayment Specific Study Plan	Three - six months prior to survey (e.g., February 15th of the survey year)
Task 2: Benthic Monitoring Surveys	Survey Summary Notification (one for each survey conducted)	August-October of survey year (e.g., August 15th of survey year)
Task 3: Analysis of Benthic Surveys	Sorting Completion Letter:	Three months after completion of survey: (e.g., November 15th of survey year)
Task 3.1: Macrofaunal Reference Collection	Reference Collection Status Letter	One month after completion of macrofaunal analysis: (e.g., December 15th of survey year)
Task 4: Synthesis Report	Synthesis Report	Draft: Four months after completion of sample sorting (e.g., March 15th of following year) Final: Two months after draft (e.g., May 15th of following year)
Task 5: Upload Data to Selected Database	Notification Email	Due after Synthesis Report and included data are approved by MassDEP
Task 6: Future Coordination with MassDEP		To be determined by MassDEP based on survey results

A7. QUALITY OBJECTIVES AND CRITERIA

Requirements for ensuring that the data are usable for their intended purpose (that is, are of suitable quality) include accuracy, precision, representativeness, comparability, and completeness. When these requirements are met, the final data product is technically defensible. Data elements for this project are discussed in terms of the appropriate characteristics, defined as:

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

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

Representativeness: The extent to which measurements represent true systems.

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

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

Quality objectives are given below. Details of how these criteria are met for each component of the Benthic Monitoring tasks are presented in Section B5.

A7.1 Field Activities

A7.1.1 *Navigation*

The quality objective for navigation is that the system used be accurate and precise to enable the sampling vessel to reliably occupy and/or re-occupy the stations that are to be sampled during the survey. Navigation equipment should be suitable for consistently fixing the vessel's position to within 10 meters. Samples will be collected within a target radius of 37 meters.

A7.1.2 *Water Quality Measurements*

The data quality goals (DQGs) for the field collection of the water quality measurements are that (1) measurements are collected before grab sampling, (2) measurements are collected at all required water depths, (3) water quality measurements will meet the accuracy, precision, and completeness requirements presented in Table 5 to be consistent with the NCCA (US EPA 2021a). Accuracy will be assessed by performing post-sampling measurements and comparing to corresponding calibration standard in accordance with manufacturer's instructions and existing Field SOP for Water Quality Measurements. Precision can be estimated from repeated measurements of samples; duplicate field measurements will be taken to assess the precision goal listed in Table 5.

Table 5. Water quality measurement accuracy, precision, and completeness data quality goals.

Measurement	Minimum Range	Accuracy	Maximum allowable Precision Goal (% Relative Standard Deviation)	Completeness
Water Temperature	-5 to +35 °C	± 1.0 °C	10%	95%
Dissolved Oxygen	0 to 15 mg/L	±0.5 mg/L	10%	95%
pH	0 to 14 units	± 0.3 units	10%	95%
Salinity	0 to 35 ppt	± 1 ppt	10%	95%
Depth	0 to 30 m	± 0.5 m	10%	95%

A7.1.3 *Grab Sampling*

The quality objectives for collection of sediment grab samples are that (1) samples be collected within 37 meters of the target location, (2) all required samples be collected, (3) samples will be of sufficient quantity to be representative of the station, (4) samples will be undisturbed, and (5) samples will be uncontaminated.

The determination of sufficient quantity is made by measuring the depth of penetration of the grab. The 0.04-m^2 Ted Young-modified van Veen grab sampler used for sediment and biological samples must contain sediment to a depth of at least 7 cm (out of a possible 10 cm) at the center. The sediment within the sampler has a relatively level surface and is not spilling out from the top of the grab (Figure 2). Procedures for collecting undisturbed and uncontaminated samples are described in Section B2.

The quality objectives for the handling of sediment samples to be used for sedimentary analysis are that (1) samples remain uncontaminated, (2) samples will be collected within the top 2.0 cm of the sediments, (3) samples be well homogenized, and (4) samples be subsampled and preserved following methods detailed in Section B2.

The quality objectives for the handling of benthic infaunal samples are that (1) samples will be handled gently during the sieving process, (2) samples will be fixed in 10% buffered formalin[2] as quickly as possible to prevent deterioration of the fauna, and (3) sample jars will be labeled accurately. Procedures for sample handling are detailed in Section B3.

All sediment samples analyzed for grain size and total organic carbon (TOC) will follow the NCCA Laboratory Analysis Requirements (US EPA 2021b, Section 7.4) as described in the MEP Benthic Monitoring Laboratory SOP (Appendix B). Samples for grain size analysis will be refrigerated at 4 °C and analyzed with any method that characterizes the sediments following Wentworth (1922) standard as described by the Coastal and Marine Ecological Classification Standard (CMECS; FGDC 2012) and meets QA/QC requirements. TOC samples will be frozen at a maximum of -20 °C and analyzed using the US EPA Method 9060.

[2] Follow safe chemical handling procedures including the use of personal protective equipment (e.g. gloves and safety glasses), the manufacture's Safety Data Sheet recommendations, and the OSHA Formaldehyde Standard (29 CFR 1910, 1048) when using this chemical.

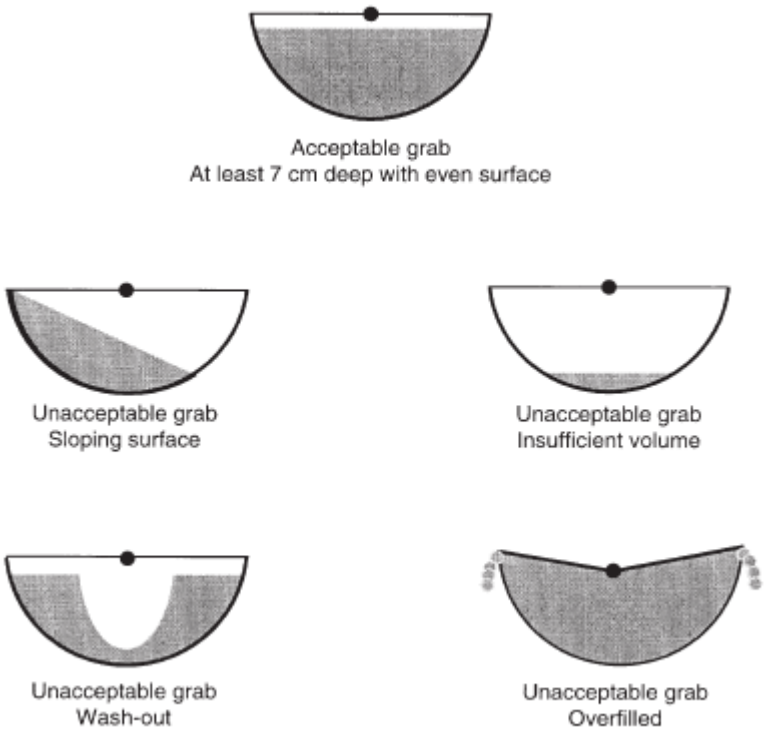

Figure 2. Illustration of acceptable and unacceptable grabs for benthic community analysis using a 0.04m² Van Veen grab sampler (from US EPA 2015a).

A7.1.4 *Underwater Digital Still Images or Video*

The DQGs for the field collection of digital still images or video are that (1) images are collected before grab sampling, (2) the images be clear and of high quality, (3) if images are collected during more than one year that they be collected from the same stations that have been sampled in previous years, and (4) all images are properly labeled and catalogued.

A7.1.5 *Sediment Profile Imagery (Optional)*

The DQGs for the field collection of the SPI are that (1) images be clear and of high quality, (2) if images are collected during more than one year that they be collected from the same stations that have been sampled in previous years, and (3) all images are properly labeled and catalogued.

A7.1.6 *Hard-bottom/Riprap Destructive Samples (Optional)*

The quality objectives for collection of destructive samples are that (1) samples be collected within 37 meters of the target location, (2) all samples required be collected, and (3) samples be of sufficient quantity to be representative of the station.

The quality objectives for the handling of destructive samples are that (1) samples will be fixed in 10% buffered formalin as quickly as possible to prevent deterioration of the fauna and (2) sample jars be labeled accurately. Procedures for sample handling are detailed in Section B3.

A7.2 Laboratory Activities

A7.2.1 *Water Quality Measurements*

Complete water quality profile data will be provided to MassDEP, therefore no analysis is required under this QAPP. If preliminary data analysis is indicated in the Embayment Specific Survey Plan (Task 1), the preliminary data analysis will meet general data validation and usability requirements in Section D.

A7.2.2 *Macrofaunal Analysis*

The DQGs for the analysis of benthic infauna and epifauna (hard-bottom destructives) are that (1) all samples be processed, (2) all animals be removed for identification and enumeration, (3) all infaunal animals be counted accurately (see Section 5.2), (4) the taxonomic identifications be accurate (correct) as discussed in Section B5.2., 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 as discussed in Section B5.

A7.2.3 *Underwater Digital Still Image or Video Analysis*

The DQGs for analysis of digital stills are that (1) at least two images from each station will be analyzed, and (2) all parameters defined in this QAPP will be analyzed for all images. The DQGs for analysis of digital videos are that (1) the required minutes of video footage (infaunal and sediment sampling = two minutes per station, or a standalone survey = 20 minutes per transect or area) will be analyzed for each station, and (2) all parameters defined in this QAPP be counted and/or measured as appropriate.

A7.2.4 *Sediment Profile Image Analysis (Optional)*

The DQGs for SPI analysis are that (1) at least three images from each station be analyzed, (2) all parameters defined in this QAPP be analyzed for all images, and (3) that analytical systems used enable repeatable measurements and determinations to be made. Accuracy and precision for SPI analysis cannot be quantified but will be optimized by QC procedures discussed in Section B5.

A8. SPECIAL TRAINING/CERTIFICATIONS

A8.1 Special Training

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

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

Instructions will be provided and documented by the Program Manager, Chief Scientist, and the Project QA/QC Officer.

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

A8.2 Certifications

Special certifications are required for the SCUBA work contained in this QAPP. All SCUBA diving conducted under this QAPP is expected to occur under a Scientific Diving Program. SCUBA divers conducting digital image surveys or hard-bottom/ riprap destructive sampling are required to have a valid and current SCUBA certification from a recognized SCUBA certification organization (e.g. PADI, NAUI, or SSI) along with first aid, CPR/AED, and Emergency Oxygen for Scuba Diving Injuries (Divers Alert Network) certifications. Divers may also have a current scientific diving certification from a recognized American Academy of Underwater Sciences (AAUS) scientific diving program.

Disclaimer: The Massachusetts Department of Environmental Protection and the Commonwealth of Massachusetts accept no responsibility and no liability for loss of any kind, including personal injury or property damage due to the work and/or activities described in this Quality Assurance Project Plan.

A9. DOCUMENTS AND RECORDS

A9.1 Documentation

Initially, all data will be recorded either (1) electronically onto computer storage media or (2) manually into bound laboratory notebooks or onto established data forms. All data collection notes will be made in permanent ink, initialed, and dated, and no erasures or obliterations will be made. If an incorrect entry is made, the information will be crossed out with a single strike mark and the correct entry will be made, initialed, and dated by the person making the correction. Corrections to electronically captured data will be documented on a hard-copy of the data. Completed data forms or other types of hand-entered data will be signed and dated by the individual entering the data. Direct-entry and electronic data entries will indicate the person collecting or entering the data. It will be the responsibility of the laboratory managers to ensure

that all data entries and hand calculations are verified according to the procedures described in Sections D1 and D2 of this QAPP.

A9.2 Field Records

Field logbooks or data forms will provide the primary means of recording the data collection activities performed during the sampling surveys. As such, entries will be described in as much detail as possible so that events occurring during the survey can readily be reconstructed after the fact. At the beginning of each survey, the date, start time, weather, and names of all sampling team members present will be entered (see Survey Log Form, Appendix A). Samples collected will be recorded on pre-printed Station Log Forms for grab and destructive samples (see Section B3.2.1), and electronically for the water quality measurements, digital still and/or video samples, and SPI.

Information specific to sample collection will include:

- Station name
- Replicate number
- Time and date of sample collection
- Sample description (color, texture, etc.)
- Samplers' initials
- Requested analyses
- Location (the geographic location where a sample is collected)

Supplementary data for every station sampled during the field surveys may be recorded in the comments section of the field data forms. For the grab samples, additional data may include notes on sampling difficulties, currents, presence/absence of anemones, and numbers and sizes of jars used for each sample.

For the SPI samples, data will be entered into an Excel spreadsheet on a laptop computer as the images are acquired. Data logged include station, date, time, sampling coordinates, number of replicates, water depth, and comments. This spreadsheet will be archived by the contractor, and a copy will be provided to the Project Manager to complete the survey logbook.

A9.3 Laboratory Records and Deliverables

Laboratory data reduction procedures will be performed according to the following protocol. All information related to analysis will be documented in controlled laboratory logbooks, instrument printouts, or other approved forms. All entries that are not generated by an automated data system will be made neatly and legibly in permanent, waterproof ink. Information will not be erased or obliterated. Corrections will be made by drawing a single line through the error and entering the correct information adjacent to the cross-out. All changes will be initialed, dated, and, if appropriate, accompanied by a brief explanation. Unused pages

or portions of pages will be crossed out to prevent future data entry. Analytical laboratory records will be reviewed by the supervisory personnel on a regular basis, and by the Laboratory QA Manager periodically, to verify adherence to documentation requirements.

Laboratories will submit data to the Project Manager as electronic data deliverables (EDD). Benthic invertebrate taxonomic laboratories will also provide copies of any hand-written data sheets. The EDDs for oligochaetes and for other benthic infauna identified under the benthic infauna tasks will be provided in Excel spreadsheets. Habitat and biotic characteristics of each station identified through digital still image or video analysis will be provided in an Excel spreadsheet. The SPI data will be provided in two versions of Excel. The first will be the original data produced by the image analysis system. The second will be reformatted according to instructions for loading into a database selected by MassDEP (e.g., the USEPA Water Quality Exchange Web [WQX Web] database). The original output will be used to ensure that the resulting files for upload to the database are correct.

Data deliverables will be provided to MassDEP by the Town's representative on the schedule described in this QAPP (Section A6.2). Details of data management are discussed in Section B10.

Sample laboratory data recording forms are provided in Appendix B.

A9.4 Reports and Data Submissions

Documents and the data submission that will be generated under the Benthic Monitoring tasks are listed below. The due dates for these reports and data submissions are tabulated in Section A6.2.

- QAPP Agreement Form (Appendix C)
- Embayment Specific Study Plan
- Survey notification emails
- Reference collection report
- Synthesis Report
- Data submission to database

A9.4.1 *Quality Assurance Project Plan (QAPP) Agreement Form*

The QAPP Agreement Form (Appendix C) will be the first document provided to MassDEP for an MEP embayment benthic monitoring program or study. Copies, either electronic or hardcopy, of this QAPP, and any subsequent revisions, will be distributed by MassDEP to the Town or teaming partner. The Town or teaming partner agreeing to follow this QAPP for an MEP Benthic Monitoring study will sign either the complete or partial implementation section, formally consenting to follow the entire QAPP or selected sections of the QAPP specific to the benthic sampling being undertaken. MassDEP will also sign the form acknowledging that the Town or teaming partner has agreed to follow the QAPP completely or partially for the specified program or study.

A9.4.2 *Embayment Specific Study Plan*

A study plan will be prepared for each embayment that benthic monitoring will be conducted in. The study plan will be submitted to MassDEP electronically at least three to six months prior to the start of sampling.

The study plan for *previously assessed* embayments will include the following information:

- Background information with a summary of conclusions from the previous assessment and current TMDL implementation and/or community measures undertaken.
- Objectives and rationale
- Selected sampling approach with specific survey methods and analytical options identified
- Schedule with sequence of tasks and events
- Specific location (map) and coordinates provided in decimal degrees latitude and longitude for each of the re-established benthic stations
- Specific location (map) and coordinates provided in decimal degrees latitude and longitude for each SPI, underwater digital image, and/or hard-bottom/riprap destructive sample station
- Survey plan - The survey plan describes in detail the scientific crew that will complete the survey work including: the Town staff and resources, consultants, and/or volunteers to be used to conduct the surveys; survey vessel, equipment and supplies; schedule of survey operations; sample handling and custody; sequence of survey tasks and events; navigation and positioning control; QA/QC procedures; and emergency contact numbers (including the phone number for the Coast Guard, and the address and phone number of the closest hospital).
- Documentation of any deviations from this QAPP

The study plan for *unassessed* embayments will include the following information:

- Description of embayment
- Specific location (map) of embayment in Massachusetts waters
- Summary of existing embayment data available
- Objectives and rationale
- Baseline sampling approach with specific survey methods and analytical options identified
- Schedule with sequence of tasks and events
- Development of an embayment-specific sampling design using a Generalized Random Tessellation Stratified approach
- Specific location (map) and coordinates provided in decimal degrees latitude and longitude for each station and type of sample collected

- Survey plan - The survey plan describes in detail the scientific crew that will complete the survey work including: the Town staff and resources, consultants, and/or volunteers to be used to conduct the surveys; survey vessel, equipment and supplies; schedule of survey operations; safety procedures (including the phone number for the Coast Guard, and the address and phone number of the closest hospital); sample handling and custody; sequence of survey tasks and events; navigation and positioning control; and QA/QC procedures
- Documentation of any deviations from this QAPP

A9.4.3 *Survey Summary Notification*

Survey summary notifications are prepared after each survey to briefly describe the sampling activities. Each notification is expected to include text containing the following information:

- An overview of the survey, including the vessel and a list of survey personnel
- Sample collection methods used during the survey
- Survey results presented as a narrative and including:
    - Actual vs. planned samples and measurements collected
    - Any unusual observations of environmental conditions
- Problems experienced, actions taken, and recommendations, including deviations from this QAPP, that were not known at the time of survey plan preparation (See A9.4.2 above).

The Chief Scientist will submit the survey summary to the Program Manager by email no later than one week after the completion of each survey.

A9.4.4 *Reference Collection Status Letter*

A reference collection letter will be prepared after macrofaunal data analysis is complete. The letter will include:

- A hierarchical taxonomic list of all taxa comprising the collection, including the station ID from which the specimen came
- The World Register of Marine Resources (WoRMS) Database ID (Aphia_ID) for each taxon
- Identification of the storage location including the address of the teaming partner or contracted laboratory and the staff with custody of the collection

A9.4.5 *Sample Analysis Data Submissions*

A9.4.5.1 *Data Submissions to the Project Manager*

Water quality data submission will include tables showing the station, depth, and value for each parameter. The macrofaunal data submissions will include tables showing the station, replicate number, taxon name, and the number of individuals counted for each taxon. The SPI

analysis data submission will be accompanied by copies of the three images that were analyzed from each site. The digital still image or video analysis data submissions will be accompanied by copies of the digital still images or video taken during the survey on an external hard drive or CDs.

The appropriate documentation (e.g., cover letter, Quality Assurance Statement), as per contract requirements (for contracted laboratories) for data submission, will be delivered to the Project Manager with the data.

A9.4.5.2 *Data Submissions by the Project Manager*

All sample analysis data will be processed into the appropriate application format as defined in the templates for or by the MassDEP selected database (e.g., WQX Web Template Dictionary). Processing of data is recommended to be completed using an advanced analytics, multivariate analyses, and data management software (e.g., R, SAS). Data processing will include error checking and checks to ensure that datasets meet database format specifications, allowable value requirements, and constraints. Data will be exported from the software (if used) in or entered into Microsoft Excel spreadsheets and uploaded into the database.

The Project Manager will notify the Town and MassDEP that the data were successfully uploaded to the database.

A9.4.6 *Synthesis Report*

The synthesis report will evaluate the sediment and associated benthic communities of the embayment for evidence of nutrient related and/or other stresses and provide a health status for the embayment.

The technical content of the report will describe the monitoring results and will provide comparisons with previous assessment if the survey was a re-evaluation. Topics will include sediment physical (i.e., grain size) and chemical (i.e., TOC) parameters and benthic infauna communities. Additional results for SPI, underwater digital imagery, and hard-bottom/riprap destructive samples will depend on the approach identified in the Embayment Specific Study Plan. These monitoring questions will be specifically addressed:

- *What is the sediment composition (e.g., sediment grain size and TOC results)?*
- *What is the habitat quality in this embayment based on qualitative images or observations?*
- *What is the characterization of the benthic community in each sub-embayment and the entire embayment based on benthic macroinvertebrate metrics (e.g., abundance, diversity, and evenness)?*
- *What is the health status of each sub-embayment and the entire embayment based on benthic macroinvertebrate metrics? If this is a reassessment, has the health status changed since the last assessment?*

- *If optional SPI was conducted, what are the observed redox depths for each sub-embayment?*
- *If optional SPI was conducted, what are the Organism-Sediment Index (OSI) scores throughout the embayment?*

A9.4.6.1 *Statistical Analyses for Sediment Data*

The sediment data will be analyzed using a variety of statistical and graphical methods. Various univariate and multivariate analyses may be employed using a standard statistical/graphics package (e.g., R, SAS, PRIMER). These tests may include analysis of variance (ANOVA), correlation analyses, or regression analyses. Additional evaluations may assess temporal and spatial trends in sediment data as compared to benthic faunal distributions.

A9.4.6.2 *Infaunal Data Analyses*

Prior to analysis of the infaunal data, some modifications to the dataset will be made. For example, some taxa, e.g., incidental pelagic faunal, encrusting, or non-benthic taxa, may be eliminated from all calculations. Other taxa may be included in calculations of abundance but not diversity; such taxa are usually those infaunal organisms that cannot be identified to species level. Only those individuals identified to species level will be included in all remaining calculations (e.g., number of species, diversity, evenness, multivariate analyses).

Multiple categories of diversity indices will be calculated: (1) species richness indices, and (2) indices based on the proportional abundances of species (e.g., Shannon-Wiener index (H'), Pielou evenness index (J'), Margalef's index, and Average Taxonomic Distinctness (AvTD; Delta+). US M-AMBI following Pelletier et al. (2018) will be used to determine sub-embayment and embayment soft bottom habitat health. US M-AMBI health condition categories include: bad (<0.20), poor (0.20 to 0.39), moderate (0.39 to 0.53), good (0.53 to 0.77), and high (>0.77). A statistical routines package (e.g., PRIMER) will be used to calculate these indices.

Cluster and non-metric multidimensional scaling analyses will be conducted in a limited manner to assess spatial and temporal trends in community composition between sites and sub-embayments. Changes in infaunal community structure between assessments may be evaluated by comparing community structure differences between stations through time, and gauging changes in community structure before and after TMDL implementation, if comparable data are available.

A9.4.6.3 *Digital Still and Video Data Analyses*

Data analysis of the still or video results will focus on two goals: (1) to obtain baseline spatial and temporal data on habitat characteristics at each station, and (2) to evaluate if observed changes, if any, in biotic parameters can be attributed to a reduction or increase in eutrophication or other embayment stressors. A minimum of two still images will be collected and reviewed from each station. If digital video is collected in place of still images during infaunal or sediment sampling, then at least two minutes will be reviewed for each station. If a

standalone video survey is conducted, then 20 minutes of video will be reviewed for each transect or area. Observations will include substrate types present, habitat types observed, flora and fauna observed, habitat relief, sediment drape, and presence of fishing or mariculture activity will be entered into an Excel spreadsheet (See Section B4.3). Data analysis products will include descriptions of habitat characteristics, species lists, and relative abundance.

A9.4.6.4 *SPI Analyses (Optional)*

A variety of statistical analyses may be used to compare SPI parameters and to display spatial variations. If SPI has been conducted before at any station, then temporal variations should also be evaluated. Analysis of variance (ANOVA) or Student's t-test for paired data may be used to test for differences between and within areas for quantitative parameters. Normality will be checked with the Shapiro-Wilk test and homogeneity of variance with Bartlett's test. If variance is not homogeneous, Welch analysis of variance, which allows standard deviations to be unequal, may be used in testing for mean differences (Zar 1999). Cochran-Mantel-Haenszel statistics and Fisher Exact Test may be used for comparisons involving categorical parameters (Agresti 1990). Statistical tests will be conducted using a statistical software program (e.g., R, SAS).

A9.4.6.5 *Hard-bottom/Riprap Destructive Data Analyses (Optional)*

Prior to analyzing the epifaunal data, some modifications to the dataset will be made. For example, some taxa, e.g., non-benthic taxa, may be eliminated from all calculations. Other taxa may be included in calculations of abundance but not diversity; such taxa are usually those immature or damaged specimens that are missing the necessary diagnostic features and cannot be identified to species level. Only those individuals identified to species level will be included in all remaining calculations (e.g., number of species, diversity, evenness, multivariate analyses).

Multiple categories of diversity indices will be calculated: (1) species richness indices, and (2) indices based on the proportional abundances of species (i.e., Shannon-Wiener index (H'), Pielou evenness index (J'), Margalef's index, and Average Taxonomic Distinctness (AvTD; Delta+). A statistical routines package (e.g., PRIMER) will be used to calculate these indices.

Cluster and non-metric multidimensional scaling analyses will be conducted in a limited manner to assess spatial and temporal trends in community composition between sites and sub-embayments. Changes in epifaunal community structure between assessments may be evaluated by comparing community structure differences between stations through time.

A9.5 Project files

The project files will be the central repository for all documents relevant to sampling and analysis activities as described in this QAPP. The Project Manager is the custodian of the project files and will maintain the contents of the project files, including all relevant records, reports,

datasheets, pictures, subcontractor reports, and data reviews in a secured, limited access area and under custody of the Project Manager.

The project files will contain at a minimum:

- Electronic copies of field and laboratory datasheets
- Embayment Specific Study Plan
- Survey Summary Notifications
- Laboratory data deliverables
- Reference collection letter
- Synthesis reports
- Data submission to database verification
- All custody documentation (chain of custody forms, etc.)

Electronic versions of reports and statistical analyses will be stored in a project-specific folder on a server or network. The original EDDs received from any laboratory contracted to conduct analyses and the project data will also be stored in the folder on the server or network and will be periodically archived. All project records will be maintained until at least the next survey (three to six years).

B. DATA GENERATION AND ACQUISITION

B1. SAMPLING PROCESS DESIGN (EXPERIMENTAL DESIGN)

The rationale for the design of the MEP benthic monitoring is provided in section A6. The benthic monitoring is designed to determine current benthic community conditions of an embayment and the long-term trends in sediment quality and benthic communities following TMDL implementation and reduction in nutrient loading to a system.

For *previously* assessed embayments: MassDEP will review the embayment technical reports for benthic sampling locations and embayment health conditions. MassDEP will select the benthic stations to be resampled for each previously assessed embayment based on 1) location in the embayment or sub-embayments, and 2) the level of habitat health with stations in embayments or sub-embayments considered "Severe Degradation" or "Significant Impairment" receiving higher priority for reassessment. Station locations and the number of samples required will depend on the sampling tier selected (Table 2). Reassessments will be conducted every three years (Tier 1) and every six years (Tier 2). Benthic infaunal sampling conducted under Tier 1 reassessments will include up to 50% of the benthic infaunal sampling locations. Reassessments conducted under Tier 2 may include reassessing all previously sampled locations; the final number of stations will be determined by MassDEP based on the results of the previous assessment. Sampling locations and sample types to be collected are embayment specific and will be identified in an Embayment Specific Study Plan (Task 1).

For *unassessed* embayments: A Generalized Random Tessellated (grid) Stratified (GRTS) survey design for an area resource will be used to determine benthic infaunal sampling locations in each unassessed embayment. This method was selected to be consistent with the US EPA NCCA program (Olsen 2010, US EPA 2015c). In general, a hierarchical square grid that covers the target population is created, the grid cells are hierarchically randomly ordered with the area of the population within each cell being used to create a line. A systematic sample is selected that identifies which cells will be sampled and a random point within the population is selected within each selected cell (McDonald 2004, Olsen et al. 2012, Kincaid 2018). For MEP marine benthic monitoring surveys, the target population will be the specific embayment selected for study following the defined NCCA estuarine resource area as described in Olsen (2010) and US EPA (2015c) with the modification of a required depth of at least 1 m at mean low water. The MassDEP MEP estuarine resources consist of all coastal waters from the head-of-salt to confluence with the ocean, including inland waterways, tidal rivers and creeks, and major embayments. The head-of-salt represents the landward or upstream boundaries. The seaward boundary extends out to where an imaginary straight-line intersecting two land features would fully enclose a coastal waterbody. All waters within the enclosed area are defined as estuarine starting at a depth of 1 m at mean low water, regardless of salinity.

The GRTS survey design will be specific to each embayment and will ensure that enough samples are collected to adequately assess the target population. Depth data to determine the

target population in a specific embayment may be available through the NOAA Electronic Navigational Charts (https://encdirect.noaa.gov/). GIS polygon layers are available for download at the 'Habor' scale band, which includes depth contours for zero meters below Mean Lower Low Water (MLLW). If depth data are not available for the entire embayment, an alternative approach is to use the MassGIS data: National Wetlands Inventory 'Estuarine and Marine Deepwater' layer (https://www.mass.gov/info-details/massgis-data-national-wetlands-inventory). This GIS polygon layer contains estuarine and marine subtidal water including open water estuary, bay, sound, and open ocean. The number of benthic sampling locations for an embayment can be based on embayment size (Figure 3). The suggested number of samples in Figure 3 should be considered an initial planning level estimate and a greater number of samples may be needed dependent on the complexity of the embayment and the sampling goals. Software to implement the GRTS survey design is available through the free software environment R project package *spsurvey*. The town(s) or teaming partner undertaking the benthic monitoring will bring a sample frame to MassDEP and coordinate with MassDEP to determine GRTS survey design and the final benthic sampling locations.

Embayment Size in Acres	Metric	Minimum Number of Samples*	Maximum Number of Samples*
< 1,750	10 sites	10	10
1,750 to 4,500	1 per 175 acres	10	26
>4500	1 per 195 acres	23	

Figure 3. Metrics to determine the number of benthic sampling locations in an embayment (* note more samples may be desired dependent on the embayment complexity and sampling goals)

The sample frame consists of the coordinates for a set of polygons that define the area resource (i.e., the specific embayment selected) with the attribute data associated with the polygons. The coordinate system for the set of points in the sampling frame will be an equal area projection so that the calculation of distance between points is valid (Kincaid 2018). The sampling frame to be used for the survey design will be in a shapefile or an s*pspurvey* package object. The type of GRTS survey design selected (i.e., stratified or unstratified, and/or equal or unequal) for an embayment will be determined in consultation with MassDEP based on the study objectives, the size and complexity of the embayment being studied, and whether or not specific areas or locations have sampling priority to address embayment specific questions (Kincaid 2018). Sampling locations and sample types to be collected are embayment specific and will be identified in Embayment Specific Study Plan (Task 1). Alternate ("over") locations will be selected to provide replacement sampling sites if a primary site could not be sampled.

A stratified sampling design divides the sampling frame into distinct groups (i.e., strata) and sites are selected within each stratum independently of the other strata. Stratified sampling allows for stratum-specific sample sizes and implementation practices (Dumelle et al. 2022). Embayments can be stratified based on several approaches including subembayments, assessment units (AU), depth, sediment type, and salinity. Stratification by subembayment should be based on historically recognized and naturally occurring subembayments, basins, and coves within an estuary system. If stratification based on depth, sediment grain size, and/or salinity is selected for use in MEP marine benthic monitoring the following descriptors will be used to define the stratum. The depth zones for the depth strata will follow the CMECS benthic depth zone modifiers (Table 6). The littoral zone is defined as all areas that are episodically exposed to air. The infralittoral zone is defined as subtidal areas within the photic zone that are often characterized by macroalgae or rooted vascular plants. The circalittoral zone is defined as subtidal areas below the photic zone and generally characterized by animal communities (FGDC 2012). Sediment grain size for the sediment strata will follow the Wentworth (1922) standard for mineral grain size definitions as adapted by CMECS (See Table 10 Section B2.2.6). Salinity zones for the salinity strata will follow CMECS salinity modifiers (Table 7). In some rare instances strata may need to be subdivided to ensure appropriate representation of the benthic communities.

Table 6. Depth zone strata (FGDC 2012).

Depth Zone	Depth Range (meters)
Littoral	Intertidal
Shallow Infralittoral	0 to < 5
Deep Infralittoral	5 to < 30
Circalittoral	30 to < 200

Table 7. Salinity strata (FGDC 2012).

Salinity Zone	Salinity Range (parts per thousand)
Oligohaline	< 5
Mesohaline	5 to < 18
Lower Polyhaline	18 to < 25
Upper Polyhaline	25 to < 30
Euhaline	30 to < 40
Hyperhaline	≥ 40

B2. SAMPLING METHODS

B2.1 Navigation

A GPS navigation system will be used to acquire navigation data for benthic monitoring surveys. Sampling will be conducted within 37 meters of the target locations as determined by the GPS navigation system (±7 meter accuracy), and the navigation data will be recorded on field station data forms (hard-copy or in Excel). If grab samples cannot be collected at the target location, they can be collected at another location within the 37 m radius of initial location. If acceptable grab samples cannot be collected within the 37 m radius, they may be taken in the >37 m to 100 m radius area of target location without the need to resample water quality. Coordinates at the location of each sediment grab sample will be recorded on the hard-copy field station log. Coordinates at the location of each sediment profile image (SPI) sample will be entered into the field station log in Excel. The vessel start and finish positions at each digital video transect or the coordinates at the location of each digital still station will be recorded on the hard-copy field station log.

For both benthic grab samples and SPI, a waypoint will be entered into the shipboard GPS when a sample is collected. The marker set for each waypoint will be named as the station name, with the replicate number appended. Waypoints will be stored separately for the grab and SPI surveys. A QC check of waypoints against the recorded coordinates will be completed after each sample is collected. Waypoints will be stored on the shipboard GPS until data checking confirms that all samples were collected within 37 meters of the target station location. Any sample coordinates found through data checking to be outside of the 37-meter station radius will be compared against the sample coordinates for the stored waypoint. Thus, if an incorrect waypoint is identified through data checking, the hand-entered data will be compared to the electronic waypoint on the GPS, and any error discovered in the navigational data will be corrected as necessary.

B2.2 Benthic Sample Collection/Shipboard Processing

All ecological sampling activities performed by the Town(s) or town representatives (Town staff, seasonal employees, volunteers, and/or contractors) for MEP benthic monitoring will be conducted following a Massachusetts Division of Marine Fisheries Scientific Collector's Permit and any local permits that are required. The Project Manager will request the appropriate permits to allow sampling; a copy will be provided to the Chief Scientist prior to the survey.

The shipboard processing and storage requirements for all samples collected for the benthic monitoring tasks are listed in Table 8 and described in the MEP Benthic Monitoring Field SOP (Appendix A). At all stations, the station coordinates, time, sea state and other weather conditions, and water depth will be recorded by hand onto a field station data form. The contracted laboratory analyzing the samples for sediment grain size and TOC will provide the sample containers for the chemistry samples. Sample containers for benthic infaunal and

epifaunal samples will be provided by the entity collecting the benthic infaunal and epifaunal samples.

B2.2.1 *Water Quality Measurements*

A multi-parameter water quality meter (or sonde) with a data recorder and temperature, dissolved oxygen, pH, and salinity/conductivity probes will be used to record water quality parameters at each station before benthic grab samples are collected. The vessel instrumentation will be used to measure the total water depth at the station to the nearest 0.1 m and the depth will be record on the Sample Collection form. The multi-parameter water quality meter will be calibrated at the start and end of each sampling day following the manufacturer's recommended procedures for each probe. The calibrations will be recorded in a Calibration Log.

The meter will be lowered into the water and single recordings will be taken at each depth once all parameters readings have stabilized. Water temperature, dissolved oxygen, pH, and salinity measurements will be collected at the following depths: 0.1 m below the surface, 0.5 m below the surface, every 1 m from depths of 1.0 to 10.0 m, and if the site is deeper than 10 m, every 5 m thereafter. Take the last set of measurements at 0.5 m from the bottom, making sure to not let the sonde touch the bottom. If the sonde touches the bottom wait for the sediment disturbance to settle for a minimum of 5 minutes. The survey crew will note any questionable measurements and retake the water quality measurements at the same depth interval as the probe is retrieved. The survey crew will note any measurements that need further comment or when a measurement cannot be made. The explanation of these notes will be placed on the Sample Collection form.

B2.2.2 *Underwater Digital Still Image Collection*

A minimum of two still images of the bottom will be collected at each station. Images will be taken perpendicular to the bottom, at a uniform distance from the surface across all samples. Turbidity can be a limiting factor; the final elevation for still images will be determined on site at the time of the monitoring based on image clarity. If divers are collecting the still images using a quadrat, a smaller quadrat may be substituted with a minimum of four images collected at each location.

The date and time will be recorded on the still image. Vessel start and finish positions, and water depth at each survey location will be captured electronically using navigational software. The date, time, station ID, vessel location coordinates, and water depth will be recorded in the field log at the start and end of each series of still image captures.

Table 8. Processing and Storage of Field Samples taken on MEP Benthic Monitoring Surveys.

Activity	Embayment Sediment and/or Infaunal Survey (Task 2.1)	Digital Still Images or Video Survey (Task 2.2)	Sediment Profile Imagery Survey (Task 2.3; Optional)	Hard-bottom/ Riprap Destructive Samples (Task 2.4; Optional)
Stations	See Survey Plan in the Embayment Specific Study Plan	See Survey Plan in the Embayment Specific Study Plan	See Survey Plan in the Embayment Specific Study Plan	See Survey Plan in the Embayment Specific Study Plan
Station location and time	Record beginning and ending location, time of station visit, and location of individual samples	Record beginning and ending location and time of station visit	Record beginning and ending location and time of station visit	Record beginning and ending location, and time of station visit
Weather/sea state/ bottom depth	Record general conditions; record bottom depth to nearest 0.5 m	Record general conditions; record bottom depth to nearest 0.5 m	Record general conditions; record bottom depth to nearest 0.5 m	Record general conditions; record bottom depth to nearest 0.5 m
Sampling: Gear	0.04-m^2 Ted Young-modified van Veen grab sampler Multiparameter sonde	Digital high-definition video camera	Digital sediment profile camera	Divers using a 1/16 m^2 frame and an airlift dredge Multiparameter sonde
Sampling: Measurements	Record penetration depth to nearest 0.5 cm and sediment volume to nearest 0.5 L Water quality profile: temperature, dissolved oxygen, salinity, pH	Record depth and heading	Record prism penetration (1 cm)	Record depth, total percent cover of macroalgae within the frame, and finfish observed in the area Water quality profile: temperature, dissolved oxygen, salinity, pH
Sampling: Sediment texture	Describe qualitatively	Not Applicable (NA)	Estimate from images (see Section B2.2.3)	NA
Sampling: aRPD depth	NA	NA	Estimate from images (see Section B2.2.3)	NA
Faunal Samples/Images: Number	3 at each station 1 water quality profile at each station	Still images – Minimum of 2 images per station Video (sediment sampling) – Minimum 2 minutes of video per station. Video (standalone survey) – 20 minutes of video per transect or area	3 at each station	3 at each station Minimum of 1 still images per replicate

Table 8. Continued.

Activity	Embayment Sediment and/or Infaunal Survey (Task 2.1)	Digital Still Images or Video Survey (Task 2.2)	Sediment Profile Imagery Survey (Task 2.3; Optional)	Hard-bottom/ Riprap Destructive Samples (Task 2.4; Optional)
Faunal Samples/Images: Processing	Rinse over 500-µm-mesh sieve; fix in 10% buffered formalin (for 48 hrs.), transferred to reagent alcohol within 7 days of collection	Digital still and/or video images save to external hard drive.	Preview images within 7 business days of survey completion (see section B2.2.3)	< 1.0 mm mesh bag; fix in 10% buffered formalin (for 48 hrs.), transferred to reagent alcohol within 7 days of collection
Faunal Samples/Images: Storage	Clean, labeled glass or plastic jar; ambient temperature	2 external hard drives	CD	Clean, labeled glass or plastic jar; ambient temperature
Chemistry Samples: Number	1 at each station (10% duplicates)	NA	NA	NA
Chemistry Samples: Processing	Use a scoop to collect upper 0–2 cm from the grab, homogenize, and collect ~50 mL subsample for grain size and ~50 mL for TOC.	NA	NA.	NA
Chemistry Samples: Storage[1]	Clean, labeled, wide-mouth glass jar (separate 125 ml [4 oz] for each grain size and TOC sample); refrigerate grain size, freeze TOC. Holding time is 28 days for both grain size and TOC.	NA	NA	NA

[1]Sediment samples will be delivered to contracted laboratories. The analysis of the total organic carbon (TOC) samples will be performed by laboratories following the US EPA Method 9060 (US EPA 2021b).

Camera lenses will be checked for debris and water droplets and carefully cleaned using a soft absorbent cloth after each time the waterproof case is opened to replace batteries or download images. In addition, in-field still image review will occur in a shaded or darkened area to help improve screen visibility and reduce glare from sunlight. A computer monitor or large screen is recommended.

B2.2.3 *Soft-Bottom Grab Sample Collection*

A 0.04-m^2 Ted Young-modified van Veen grab sampler will be used to collect bottom sediment samples for grain size, TOC, and infaunal analysis. At each station three grab samples will be

collected for macrofaunal analysis (two sorted and one archived) and one grab sample will be collected for chemical analyses. Grab samples will not be collected at stations where eelgrass (*Zostera marina*) is present.

Once the survey vessel is on station and coordinates have been verified, the water quality and underwater video surveys will be performed (see Appendix A Sections II and IV). Once these surveys are complete, the sediment grab will be deployed. When slack in the winch wire indicates that the grab is on the bottom, the grab and captured sample will be brought back to the surface. Upon retrieval of the grab, the sample will be inspected for acceptability (see Section A7.1.2). If the sample is unacceptable, the grab will be emptied, rinsed, and redeployed.

If the sample is acceptable, the penetration depth, sediment volume, and sediment texture will be visually estimated. The volume of the grab will be estimated by comparing the measured penetration depth with a prepared table of penetration depths versus grab volumes (Table 9). These data will be recorded in the field log. A field description of sediments is required following measurement of penetration depth. The sediment should be characterized as being coarse sand, fine sand, silt, clay, gravel, mud, or of a mixed type. The presence of shell hash should also be recorded. Obvious odors, such as hydrogen sulfide (the odor of rotten eggs), petroleum, other odors, or a lack of noticeable odors should be recorded. General sediment colors (i.e., black, green, brown, red, or gray) should also be recorded.

For the infaunal samples only, after these measurements are taken, the grab will be placed over a bucket, the jaws opened, and the sample emptied into the bucket. Ambient seawater will be used to gently wash the sample into the bucket. Once thoroughly washed, the grab will be redeployed until the required numbers of acceptable samples have been obtained for infaunal analysis.

Precautions will be taken during the deployment and retrieval of the grab sampler to prevent contamination of samples between stations. Sampling for grain size, TOC, and infauna determinations require that the grab and associated sampling equipment be washed with ambient seawater. To estimate the overall precision or repeatability of results, a subset of field samples are replicated by taking co-located (a new location within 37m radius around the site) and sequential duplicate samples. At a minimum, 10% of the total number of samples group is typically collected as a duplicate. For this project, a minimum of 10% of grain size and TOC sediment samples will be collected as a duplicate.

Table 9. Values used to convert Grab Penetration Depth to Sediment Volume.

Grab Penetration Depth (cm)[1]	Sediment Volume (L) 0.04-m^2 Grab
4.1-5.0	1.4
5.1-6.0	1.8
6.1-7.0	2.1
7.1-8.0	2.4
8.1-9.0	2.7
9.1-10.0	3.0
10.1-11.0	
11.1-12.0	
12.1-13.0	
13.1-14.0	
14.1-15.0	

[1]Over penetration is > 9.5 cm for 0.04-m^2 grab.

B2.2.4 *Grab Sample Shipboard Processing*

At grab stations, grab samples for infaunal analyses will be rinsed with clean seawater through 500-µm-mesh sieve. The portion retained on the screens will be transferred to labeled jars and fixed in 10% buffered formalin. Sample jars will be glass, Nalgene, or other sturdy plastic jars with screw-capped lids. Each sample jar will be filled no more than half full of material and filled to within 1 cm of the top with seawater to prevent infauna from sticking to the sample jar top. The jar will be gently turned around on its side to distribute the formalin evenly throughout the sample. The technician sieving each sample will be identified by his or her initials in the survey log. Sieves will be washed between samples. Sample jars will be labeled with external and internal labels. The lids on the sample jars will be taped and the jars inserted individually into large zip-locked or tied plastic bags lined with absorbent padding (Section B3.1, Appendix A)

If the grab sample to be used for sediment analyses meets the acceptability criteria, the water overlying the sample will be siphoned from the grab and the surface sediment (top 0 to 2 cm) will be collected with a scoop and transferred to a clean (rinsed with clean seawater) glass bowl. The sediment will be thoroughly homogenized before being transferred to appropriate storage containers. Approximately 50-mL subsamples for grain size analysis and 50- mL subsamples for TOC will be placed into separate 125 mL (4 oz) wide-mouth sample jars. These samples will be labeled and refrigerated at 1 to 4°C. These samples will be delivered to the contracted laboratory for analysis within 24 hours of survey completion.

The maximum holding time for sediment samples will be 28 days. The U.S. EPA has suggested holding times for various sample matrices and analyzes (Kahn 1988, US EPA 1992, 2016a). This

time frame is consistent with standard EPA Methods and assures that samples are analyzed in a timely manner to prevent or minimize analyte degradation and interferences.

B2.2.5 *Underwater Digital Video Collection (Optional)*

For a stand-alone video survey, at least 20 minutes of high-definition video footage will be obtained from each station or area. If transects are established and conducted using a camera system towed from a vessel, the vessel will move at approximately ¼ knot speed during video recording to maintain position along the transect. Slow vessel speed will enable clear, detailed, and reviewable video images to be obtained. If divers or a remotely operated underwater vehicle (ROV) are used to conduct the survey, the survey vessel will be anchored at the station to maintain position. The video camera system will be held approximately one meter (2 to 4 feet) off the bottom to attain a field of view of one square meter. As turbidity can be a limiting factor, in areas of poor visibility the video camera system can be lowered to maintain image clarity. The image clarity will be paramount in determining the final elevation. Simultaneous high definition still images can be captured using the same camera depending on the available settings or small digital camera (e.g., GoPro) in a waterproof housing attached the frame or sled throughout the survey at each station (see Section B2.2.3).

The date and time will be recorded on the video image. Vessel start and finish positions, and water depth at each survey location will be captured electronically using navigational software. The date, time, station ID, vessel location coordinates, and water depth will be recorded in the field log at the start of each video recording, and at the end of each video recording.

Camera lenses will be checked for debris and water droplets and carefully cleaned using a soft absorbent cloth after each time the waterproof case is opened to replace batteries or download images. In addition, in-field video review will occur in a shaded or darkened area to help improve screen visibility and reduce glare from sunlight. A computer monitor or large screen is recommended.

B2.2.6 *Sediment Profile Image Collection (Optional)*

SPI is an option for consideration in larger embayments (e.g., all of Buzzards Bay or Cape Cod Bay) or coastwide assessments to provide a rapid evaluation of the structure and appearance of embayment surface sediments. A sediment profile camera can sample stations arranged in transects or grids in rapid succession. SPI is therefore useful for baseline mapping of seafloor physical and biological characteristics, delineating areas affected by hypoxia or anoxia, identifying organic enrichment gradients, and documenting benthic habitat types across large areas (Germano et al. 2011). SPI can be used on its own or in conjunction with other sampling and mapping techniques, including benthic and sediment grab sampling, side-scan sonar, and multibeam or swath bathymetry. SPI is best used as a screening tool to map gradients in physical, biological, and chemical processes (Germano et al. 2011).

The sediment profile camera system consists of a digital camera (e.g., Canon 7D, 18-megapixel sensor) enclosed in a pressure-resistant housing, a 45° prism, and a mirror that reflects an image of the sediment through the camera lens. A strobe mounted inside the prism is used to illuminate the sediment. The prism is also equipped with a video camera with a feed to the surface via cable so that prism penetration can be monitored in real time. The camera/prism system is mounted in a cradle that is secured to a larger frame, which ensures that the prism penetrates the sediment at a 90° angle. In addition, the camera frame supports a plan-view video camera mounted to view the surface of the seabed in front of the prism. Prior to every field deployment, all essential items are gathered and tested for proper operation.

The entire assembly is lowered by winch at a steady rate to the seafloor. Images from the video-plan camera are relayed to the surface via the video cable and permit the camera operator to see the seafloor and know when the camera has reached the bottom. The camera operator can then view the prism penetration and choose when to record sediment profile images. A series of two-four photographs will be taken, each time the camera is on the bottom, generally within the first 12 seconds after bottom contact. This sampling protocol helps to ensure that at least one usable photograph is produced during each lowering of the camera. After the required number of replicates, the camera assembly is returned to the ship. The date, time, station, replicate, vessel location, water depth, and comments will be recorded in a field log with each touchdown of the camera.

The digital camera saves images to compact flash solid-state memory cards. The video signal (from the plan-view video camera) is recorded on mini-DVD digital videotape for later review. The combination of video and digital images will ensure accurate and reliable collection of SPI data. The video contributes the real-time assessment component, whereas the still images provide high-resolution detail for full image analysis in the laboratory.

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 Coastal and Marine Ecological Classification Standard (CMECS) sediment grain size descriptors (Table 10)
- Sediment layering, thickness, and type
- Surface and subsurface fauna and structures
- Approximate prism penetration
- Approximate surface relief
- Approximate aRPD - categorized following the CMECS depth modifiers (Table 11)
- Other major, readily discernable patterns

Within one week of completion of the rapid review, the results will be communicated to the Program Manager via an e-mail summary of the survey.

Table 10. 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 11. 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

B2.2.7 *Hard-Bottom/Riprap Destructive Sample Collection (Optional)*

A hard bottom/riprap survey is an option for consideration when (1) an embayment has a large amount of hard substrate (e.g., cobble, boulder, or ledge) where grab sampling will not adequately characterize the benthic communities or (2) one of the study objectives is to characterize the habitat provided by the embayment's shoreline armoring.

Destructive samples will be collected by divers using a 1/16 m^2 frame placed horizontally on the hard substrate and an airlift dredge. The first replicate is located by going from the starting location along a specified compass azimuth for the specified kick strokes. A kick stroke is defined as a kick of the right foot in a normal kicking cycle and is closely equivalent to a distance of 1 meter. Care will be taken to avoid kelp and mussel beds. The diver will take a digital image with an underwater camera and note the total percent cover of macroalgae found within the frame and any finfish observed in the area before sample collection. The sample will

then be collected into a mesh bag with a mesh size of less than 1.0 mm. The square is to be vacuumed at a high suction rate to capture the highly motile organisms, then large organisms such as echinoderms and algae will be picked by hand and placed into the bag. The area within the frame will then be cleaned of all organisms including all crevices and rock sides to a depth of 4 inches. The crustose algae should be chipped to expose pockets where animals may reside. When sampling is completed, the bag is removed, tied, and placed in a plastic bag. The diver will squeeze as much water as possible from the plastic bag before tying it off. The bag will then be placed in a yellow mesh bag and secured to the airlift or the mooring block. The diver must match the mesh bag with the proper internal tag that identifies the azimuth of the sample. Scraped surface type is recorded on the Field Data Sheet after the sample is collected. This process is repeated for each replicate. After the remaining replicates are sampled, the samples are returned to the surface. Samples will be placed in 500 mL sample jars, fixed with 10% buffered formalin, and labeled. The jar will be gently turned around on its side to distribute the formalin evenly throughout the sample.

B2.3 Unanticipated Changes to Sampling Methods

The Chief Scientist must notify the Project Manager and MassDEP, Town or teaming partner before any unanticipated changes to the survey sampling methods occur. Examples of unanticipated changes include, but are not limited to, if an unanticipated barrier, mooring field, or eelgrass is encountered, any changes to sample locations or transects, and/or length and proximity of video sites must be discussed with the Project Manager prior to sampling. This notification requirement will ensure that the survey will be conducted as stated in the Survey Plan and the selected procedures from the Field SOP unless there is a compelling reason for modification. The notification will also facilitate a brief discussion between the Chief Scientist, Project Manager, and MassDEP, the Town, or teaming partner which will help ensure that possible consequences in data collection and data quality are considered.

B3. SAMPLE HANDLING AND CUSTODY

B3.1 Sample Handling

Handling of samples while in the field, including storage requirements, is described in Section B2.2 (see Table 8) above.

Following the soft-bottom benthic survey, the infaunal samples (stored in sturdy coolers) will be delivered by a survey crew member to the contracted laboratory. A crew member will contact laboratory staff to arrange a time for sample drop off. This will allow laboratory staff to prepare for sample receipt. The samples, while still in buffered 10% formalin, can be shipped by ground or two-day express delivery if necessary for delivery to contracted laboratory. The lids on the sample jars will be taped and the jars inserted individually into large zip-locked or tied plastic bags lined with absorbent padding. At the laboratory, one sample from each infaunal station will be randomly selected to archive (see Section B3.2.3) and the other two will be

processed. The samples will be fixed in buffered 10% formalin for at least 48 hours, and then be transferred to reagent alcohol within seven days of collection. The organisms will be picked from the samples and sorted into major taxonomic groups.

The sediment chemistry samples collected during the benthic survey must be kept cold or frozen as described in Table 8. After the survey is completed, a survey crew member will deliver the sediment chemistry samples to the contracted laboratory. A crew member will contact laboratory staff to arrange a time for sample drop off. This will allow laboratory staff to prepare for sample receipt. The survey team will keep the laboratory informed about any changes to the expected delivery time. All samples will be kept on ice in coolers during transport. If circumstances dictate that the samples must be shipped to the laboratory, they will be shipped by overnight express. In that case, the samples that were frozen after collection will be placed on dry ice with protective layers of foam or bubble wrap to ensure that they remain intact and frozen during shipment.

Following the hard-bottom destructive sample survey, the samples (stored in sturdy coolers) will be delivered by a survey crew member to the contracted laboratory. A crew member will contact laboratory staff to arrange a time for sample drop off. This will allow laboratory staff to prepare for sample receipt. The samples, while still in buffered 10% formalin, can be shipped by ground or two-day express delivery if necessary for delivery to contracted laboratory. The lids on the sample jars will be taped and the jars inserted individually into large zip-locked or tied plastic bags lined with absorbent padding. At the laboratory, one sample from each infaunal station will be randomly selected to archive (see Section B3.2.3) and the other two will be processed. The samples will be fixed in buffered 10% formalin for at least 48 hours, and then be transferred to reagent alcohol within seven days of collection. The organisms will be picked from the samples and sorted into major taxonomic groups.

B3.2 Sample Custody

B3.2.1 *Sample Tracking*

Sample custody will be tracked through external and internal sample labels (Figure 4), sample collection forms (Figure 5), and chain of custody (COC) forms (Figure 6).

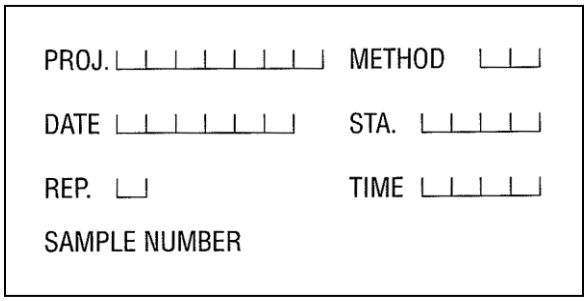

Figure 4. Example of a Macrofaunal Sample Label.

Sample Collection Form: Soft-Bottom Infaunal and Sediment Grab Samples

MEP Project Name:

STATION: ________________ TIME ON STATION:__________________ STATION DEPTH (M):__________________DATE:_____________________	Weather:_________________________ Recorded By:_________________________
Sample data	**Field Measurements**
Sample ID:	Grab Size: 0.04-m^2
Latitude:	Grab Penetration (cm):
Longitude:	Sediment Texture:
Replicate:	Other:
Time:	Analyses: (circle all applicable) GR TOC FA
Sieved By:	Organisms observed:
Comments:	
Sample ID:	Grab Size: 0.04-m^2
Latitude:	Grab Penetration (cm):
Longitude:	Sediment Texture:
Replicate:	Other:
Time:	Analyses: (circle all applicable) GR TOC FA
Sieved By:	Organisms observed :
Comments:	
Sample ID:	Grab Size: 0.04-m^2
Latitude:	Grab Penetration (cm):
Longitude:	Sediment Texture:
Replicate:	Other:
Time:	Analyses: (circle all applicable) GR TOC FA
Sieved By:	Organisms observed :
Comments:	
Sample ID:	Grab Size: 0.04-m^2
Latitude:	Grab Penetration (cm):
Longitude:	Sediment Texture:
Replicate:	Other:
Time:	Analyses: (circle all applicable) GR TOC FA
Sieved By:	Organisms observed :
Comments:	
GR = grain size, TOC= total organic carbon, FA = Infauna	

Figure 5. Example of a Sample Collection Form.

Chain of Custody Form

Project Name: _____
Project Number: _____
Originating Contact: _____
Originator Location: _____
Final Destination: _____
Sampler(s): _____

Sample		Collection		Containers		Grab.	Comp.	Presv.		Parameters				Page of
No.	Identification	Date	Time	No.	Type									Method of Shipment:
														Comments Below:
				Total										

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:
Date:	Date:	Date:	Date:	Date:	Date:

Figure 6. Example of a Chain-of-Custody Form.

Sample information for media generated by digital still image and/or video surveys (station, date, cruise) will be electronically labeled, and a placard with station information will be photographed before each transect or quadrat image.

Sample labels for macrofaunal samples will be affixed to the sample containers in the field. The sampling station, sample type, replicate number, date and time will be entered manually onto the label. One additional label will be prepared on ascot paper and inserted inside the sample container. If multiple sample containers are needed for a single infaunal replicate, the sample information will be manually entered on blank labels, and the containers will be numbered (e.g., "1 of 2", "2 of 2"). Sampling station and replicate number will be printed on the COC forms.

Sediment samples collected under this QAPP will be processed by a contracted laboratory. The contracted laboratory will provide the sample containers and sample labels (Figure 7). Sample labels will contain or have spaces for the following information: station location, survey type, analysis, preservative, date/time collected, and collected by. The Chief Scientist is responsible for verifying sample information on the sample labels matches the information on the COC forms prior to delivering the samples to the contracted laboratory.

The survey crew will fill out the sample collection form (Figure 5) at each station. The form includes header fields for entering pertinent information about each station, such as arrival time, bottom depth, and weather observations. In addition, the form sheets contain spaces for specific grab data, such as penetration depth and general descriptions. These sheets will remain in the survey logbook and will be maintained in the project files. During field collection, COC forms (Figure 6) will also be completed. The COC forms will include the unique information from the corresponding label on the sample container, ensuring the tracking of sample location and status.

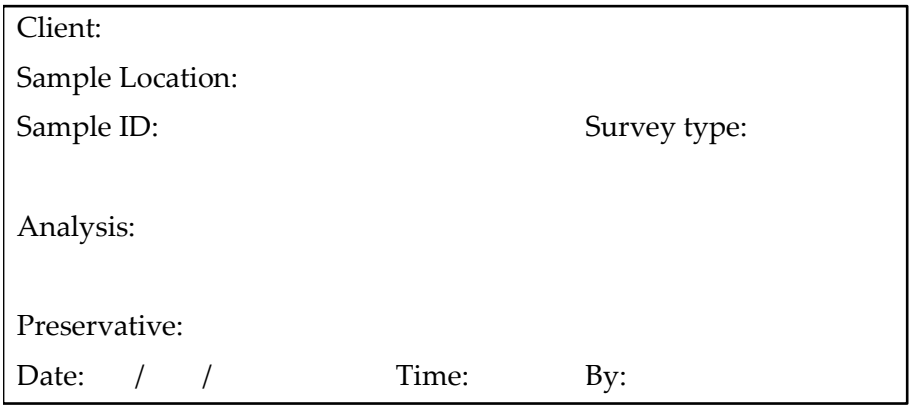

Figure 7. Example of a Sediment Sample Label.

B3.2.2 *Sample Custody*

Macrofauna and sediment samples will be in the custody of the survey Chief Scientist or a crew member from collection until they are transferred to the contracted laboratory. COC forms (Figure 6) will accompany the samples. One complete (copied) set of the macrofauna COC forms will be included in each shipping container and the original COC forms will be returned to Project Manager after the samples have been logged in at the contracted laboratory. The signed original custody forms will be retained in the project files. Sample processing will occur in the contracted laboratory. After the samples are processed, the laboratory will store the appropriate samples and specimens for the specific length of time for re-identification QC, voucher, or unforeseen circumstances.

Transfer of benthic infaunal, sediment, and hard-bottom destructive samples will be documented on the custody forms. All samples will be distributed to the appropriate laboratory personnel by hand or by a shipping service. A copy of the COC form will be retained by the field sample custodian in the field log. The original will accompany the samples to the laboratory for subsequent sample transfer. When samples arrive at the laboratory, custody will be relinquished to the laboratory staff. The laboratory staff will verify that the custody seals on the cooler are intact. The laboratory staff will then examine the samples, verify that sample-specific information recorded on the COC is accurate and that the sample integrity is uncompromised, log the samples into their laboratory tracking system, and complete and sign the COC form so that transfer of custody of the samples is complete. Any discrepancies between sample labels and transmittal forms, the condition of the samples upon receipt, and any unusual events or deviations from the QAPP will be documented in detail on the COC, and the Project Manager notified. Copies of completed custody forms will be delivered (scanned and emailed or faxed) to the Project Manager within 24 hours of receipt.

All original data from the digital stills and/or digital video will be generated and maintained by the Chief Scientist. If the survey Chief Scientist is only responsible for obtaining but not analyzing the data, then digital still and/or video data will be delivered to the Project Manager within 1 week of survey completion.

All original SPI field data sheets and associated media (video and digitally formatted media) will be generated by and remain in the custody of the Chief Scientist. If the survey Chief Scientist is only responsible for collection of the data and not data analysis, then field data sheets and associated media will be delivered to the Project Manager within one week of survey completion.

B3.2.3 *Sample Archival Policies*

The types of materials that may be archived under this project include samples, sample residues, a reference collection, and other infaunal specimens.

One randomly selected sample from each soft-bottom infaunal station or hard-bottom/riprap destructive station will be archived, and the other two will be processed. Archived soft-bottom infaunal samples will be rinsed with fresh water over 500-µm-mesh screens and transferred to reagent alcohol for storage at the laboratory. Archived hard-bottom/riprap destructive samples will be rinsed with fresh water over a 1.0-mm-mesh screen and transferred to reagent alcohol for storage at the laboratory.

Macrofauna samples (both archived and processed samples) 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 QC procedures are completed and the data are accepted by the Project Manager, then the residues may be discarded. Reference collection specimens will be retained by the contracted laboratory until the next survey (3 to 6 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, and stored under appropriate conditions for the length of the storage period. Other macrofauna specimens may be retained by the contracting laboratory indeterminately as there is no contractual obligation regarding those specimens.

B4. ANALYTICAL METHODS

The parameters to be measured during the various benthic monitoring tasks can be characterized as macrobiological and sedimentological (habitat properties; Table 12). Macrobiological parameters are based on (1) the species-level identifications of the soft-bottom infauna or hard-bottom epifauna and (2) identifications of epibenthic macrofauna seen in the underwater digital images; these parameters include community measures such as abundance (or percent cover), numbers of species, and diversity. SPI studies (optional) measure the general nature of the infaunal community, which also generate information about sediment geophysical properties, including sediment grain size and total organic carbon.

Table 12. Benthic Survey Sample Analyses.

Parameter	Unit of Measurement	Method	Reference
Infaunal Analysis	Count/species (# per grab)	ID and Enumeration	Section B4, this QAPP; Appendix B
Underwater Digital Images	Various	Various	Section B4, this QAPP; Appendix B
Sediment Profile Images (Optional)	Various (see Table 13)	Various	Section B4, this QAPP; Appendix B
Hard-bottom Destructive Sample (Optional)	Count/species (# per sample)	ID and Enumeration	Section B4, this QAPP; Appendix B
Physicochemical Parameters			
Sediment Grain Size	% dry weight	Folk 1974, FGDC 2012	Appendix B
TOC	%C by dry weight	US EPA Method 9060	US EPA 1986

B4.1 Soft-bottom Infaunal Analysis

Samples will be rinsed with fresh water over 500-µm-mesh screens to remove any broken-up mud casts and transferred to reagent alcohol for storage prior to sorting. To facilitate the sorting process, all samples will be stained in a saturated alcoholic solution of Rose Bengal at least overnight, but no longer than 48 hours to avoid over-staining. After rinsing with clean fresh water, small aliquots of the sample will be placed into white plastic or enamel pans, and all organisms, including anterior fragments of polychaetes, will be removed and sorted to major taxonomic categories such as polychaetes, arthropods, and mollusks. Sorting will be done under a dissecting microscope, and organisms will be placed into vials of denatured 100% reagent alcohol (Appendix B). If samples contain an extremely high number of individuals that cannot be easily separated from the sediment matrix (e.g., juvenile bivalves approximately 0.5 mm in size), then the samples may be subsampled (see Section B4.1.1 below and Appendix B).

After samples have been completely sorted, the organisms will be delivered to taxonomists for identification and enumeration. Identifications will be made to the lowest practical taxonomic level, usually species (Appendix B). For oligochaetes, only whole organisms or fragments that include the head will be counted and identified to the taxonomic level of Class. Nematodes will not be counted for infaunal analysis under this QAPP. Immature or damaged specimens that are missing the necessary diagnostic features for identification to the species taxonomic level will be identified to the lowest practical taxon. To ensure consistency for assessment of the soft-bottom macrofaunal community, any incidental pelagic organisms or fauna attached to hard-substrates will not be identified.

Infaunal data will be recorded on project-specific data sheets (Appendix B) and will then be entered into an electronic format. Data entered into an electronic database will either be manually verified for accuracy or will be entered in duplicate, and a comparison program run to identify any discrepancies.

A project-specific reference collection will be established, which will be used by project taxonomists to ensure comparability of the taxonomic identifications performed between surveys and laboratories. This collection will be maintained by the laboratory performing macrofauna taxonomic identifications and will be checked regularly by staff to ensure that it is stored properly to reduce the risk of alcohol evaporation and damage, and to ensure that labels are intact and legible. Vials in which the reagent alcohol level is low will be filled with clean alcohol. Any labels showing signs of deterioration will be replaced.

As taxa not previously identified during the program are encountered, they will be added to the collection. As part of the maintenance of the reference collection, taxonomists will review any possible inconsistencies between previous identifications and those made during the current survey. The taxonomic status of species in the collection will be evaluated as relevant systematic revisions appear in the scientific literature. If necessary, recommendations for changes in taxonomic usages will be made to MassDEP. The reference collection will be maintained until the next survey (three to six years). The collection will be sent to the Project Manager of the next survey or another designated laboratory. If the next survey does not occur by the end of six years, MassDEP will be contacted and consulted on collection disposal.

Additional details on infaunal sample analysis methods that are not specified elsewhere in this QAPP are provided in the MassDEP MEP Benthic Monitoring Laboratory Standard Operating Procedures (Appendix B). For any case in which the Laboratory Standard Operating Procedures are different from those described in this QAPP, the procedures described in this QAPP will be followed.

B4.1.1 *Subsampling*

During the sorting process, if a sample contains organisms that are not separable from the sediment, then an additional step of subsampling may be warranted. For example, in the Pilot Study: Pleasant Bay (Sweeny and Rutecki 2019), an extremely large number of very small juvenile bivalves were essentially the same size as the sieve (0.5 mm) and therefore could not be practicably counted. The approved solution for this example was to subsample the material that remained on the 0.5 mm mesh. This method may also apply to other organisms and substrates. All subsampling will need prior discussion and approval of the Project Manager, MassDEP, and/or the Town/client.

For samples that have been approved for subsampling, first decant the reagent alcohol from the sample container by pouring the fluid through a 0.5 mm sieve into a separate container. Gently wash the sample material and separate any light material and organisms from the heavy

material. The light material and organisms will be set aside and sorted following the standard process above. Separate the heavy material into homogeneous sizes to facilitate sorting using a stack of different sized sieves finishing with a 0.5 mm sieve at the bottom of the stack. Material on the different sized sieves that does not require subsampling will be set aside and sorted following the standard process. The material that is to be subsampled will be placed into a pan marked with a grid (for example 30.5 cm by 30.5 cm [12 in by 12 in] inside dimensions with marks at the edges for grids 6 cm by 6 cm). The sample material will be spread over the bottom of the pan as evenly as possible. Use a random number generator to select at least 10% (or the approved percentage) of the squares to be processed for subsampling to ensure that the subsample material is representative of the overall sample. For example, if the tray is subdivided into 30 squares, then 3 squares would be selected for sorting. Remove all material from the selected squares and place the subsampled material into a sorting tray and proceed with sorting following the standard process above. Record the number of organisms found in the subsampled material on the Sample Card or datasheet. Remove the remaining material left in the sorting pan from the selected squares (i.e., sticks or organic debris) and place it in a separate container with reagent alcohol, labeled "Picked". Return all material not subsampled to the original container with preservative and label "Unsorted Sample Remains". Raw counts from the subsampled material will be multiplied by the appropriate factor, based on the approved percentage of grid squares sampled, to achieve the estimate for the entire sample. Additional details on the subsampling method are provided in the MassDEP MEP Benthic Monitoring Laboratory Standard Operating Procedures (Appendix B).

B4.2 Underwater Digital Images Analysis

B4.2.1 *Digital Still Images*

Digital still images collected at the start of soft-bottom infaunal grab samples or hard-bottom/riprap destructive sampling will be used as a visual record of the bottom habitat at the sampling location. These images will only be analyzed as described in the following paragraph if specifically stated in the Embayment Specific Study Plan.

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 square meter area using a photograph editing software (e.g., Adobe Photoshop). Substrate types will be characterized for particle size following the CMECS sediment grain size descriptors (Table 10). Additional substrates that could be observed in the digital images are discussed in Table 13. Habitat relief will be characterized as none, low, moderate, and high as described in Table 14. Drape will be characterized as absent, low, moderate, or heavy as described in Table 15. 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 placing 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 made based on the descriptions in Table 16.

B4.2.2 *Digital Video*

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 by particle size following the CMECS for sediment grain size (Table 10). Additional substrates that could be observed in the digital images are described in Table 13). 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 14). Drape is the visible layer of detrital material on the top of rock surfaces composed of fine-grain sediment, phytodetritus, zooplankton fecal pellets, tubes, and mucus. Drape will be characterized as absent, low, moderate, or heavy (Table 15). 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 be made based on the descriptions in Table 17. 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 reviewed 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. This spreadsheet will be delivered to the Project Manager for submission to MassDEP and the selected database.

Table 13. 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).
Crepidula Reef Substrate	Shell Reef that is primarily composed of conglomerated *Crepidula* 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 14. Habitat relief descriptions.

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

Table 15. 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
Heavy	Most of the hard substrate or encrusting/fouling organisms are covered and indistinguishable

Table 16. 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

Table 17. 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

B4.3 Sediment Profile Image Analysis (Optional)

After field collection, SPI analysis will continue with a reanalysis of the plan-view video previously examined in the field (section B.2.2.3). A visual analysis including the same parameters as estimated from the video SPI will be made for the still images. The final rapid "quick look" analysis based on this review of both video and still images will be completed within seven days of the completion of field work.

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 pre-formatted, standardized spreadsheet file. The parameters to be measured are summarized in Table 18 and discussed in more detail in Appendix D. Further details about these analyses can be found in Rhoads and Germano (1982, 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 (e.g., Adobe Photoshop™) and image processing programs (e.g., National Institutes of Health ImageJ) are used to preprocess

and analyze the images. Computer analysis of each image is standardized by executing a series of macro commands. SPI results, in the form of an Excel spreadsheet, will be delivered to the Project Manager for checking and submission to MassDEP.

Table 18. 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	—	V	Count
Feeding (Oxic) Voids	Number	V	Count
• Successional Stage	—	V	Identify
Organism-Sediment Index (OSI)	—	CA	Derived from aRPD, Successional Stage, and Voids (Rhoads and Germano 1982, 1986)

[1] V: Visual measurement or estimate
CA: Computer analysis

B4.4 Hard-bottom/ Riprap – Destructive Sample Analysis (Optional)

Samples will be rinsed with fresh water over 0.5 mm-mesh screens and transferred to reagent alcohol for storage prior to sorting. To facilitate the sorting process, all samples will be stained in a saturated alcoholic solution of Rose Bengal at least overnight, but no longer than 48 hours to avoid over-staining. After rinsing with clean fresh water, small aliquots of the sample will be placed into white plastic or enamel pans, and all organisms, including anterior fragments of polychaetes, will be removed and sorted to major taxonomic categories. Sorting will be done under a dissecting microscope, and organisms will be placed into vials of denatured 100% reagent alcohol (Appendix B).

After samples have been completely sorted, the organisms will be delivered to taxonomists for identification and enumeration. Identifications will be made to the lowest practical taxonomic level, usually species (Appendix B).

Epifaunal data will be recorded on project-specific data sheets (Appendix B) and will then be entered into an electronic format. Data entered into an electronic database will either be manually verified for accuracy or will be entered in duplicate, and a comparison program run to identify any discrepancies.

A project-specific reference collection will be established, which will be used by project taxonomists to ensure comparability of the taxonomic identifications performed between surveys and laboratories. This collection will be maintained by the laboratory performing macrofauna taxonomic identifications and will be checked regularly by staff to ensure that it is stored properly to reduce the risk of alcohol evaporation and damage, and to ensure that labels are intact and legible. Vials in which the reagent alcohol level is low will be filled with clean alcohol. Any labels showing signs of deterioration will be replaced.

As taxa not previously identified during the program are encountered, they will be added to the collection. As part of the maintenance of the reference collection, taxonomists will review any possible inconsistencies between previous identifications and those made during the current survey. The taxonomic status of species in the collection will be evaluated as relevant systematic revisions appear in the scientific literature. If necessary, recommendations for changes in taxonomic usages will be made to MassDEP. The reference collection will be maintained until the next survey (three to six years). The collection will be sent to the Project Manager of the next survey or another designated laboratory. If the next survey does not occur by the end of six years, MassDEP will be contacted and consulted on collection disposal.

Additional details on macrofaunal sample analysis methods that are not specified elsewhere in this QAPP are provided in the MassDEP MEP Benthic Monitoring Laboratory Standard Operating Procedures (Appendix B). For any case in which the Laboratory Standard Operating Procedures are different from those described in this QAPP, the procedures described in this QAPP will be followed.

B5. QUALITY CONTROL

B5.1 Sampling

B5.1.1 *Navigation*

Accuracy and Precision

A GPS navigation system will be used to acquire navigation data for soft-bottom benthic surveys. Hand-entered coordinates for infaunal grab, SPI, underwater digital images, and hard-bottom destructive sample locations will be checked against electronic waypoints on the shipboard GPS after each sample is collected. Waypoints will then be stored on the shipboard GPS until data checking confirms that all samples were collected within 37 meters of the target station location. Any incorrect waypoint that is identified through data checking will be corrected using the electronic waypoint stored on the shipboard GPS.

Comparability

All sampling locations will be comparable to locations obtained in previous monitoring activities for assessed embayments or the Embayment Specific Study Plan for unassessed embayments as well as by other researchers that have used or are using GPS at these stations. The sampling locations listed in the Embayment Specific Study Plan are targets and at each station the vessel is positioned as close to the target coordinates as possible. For the underwater digital video surveys, the start and end points of each transect are recorded.

Completeness

For all navigation data, 100% completeness has been defined as the QAPP requirement. GPS navigation systems will be used to acquire navigation data for all surveys. Depth measurements will be recorded at each station. The Chief Scientist will review station logs prior to leaving each station to ensure that these data have been accurately collected.

B5.1.2 *Water Quality Measurements*

Accuracy and Precision

A multi-parameter water quality sonde will be used to record the specified water quality parameters. Accuracy and precision of water quality measurements will depend on following the manufacturer's recommended maintenance and calibrations of the sonde. Accuracy will be assessed by performing post-sampling measurements and comparing to a corresponding calibration standard in accordance with manufacturer's instructions and existing Field SOP. Precision is estimated from repeated measurements of samples; duplicate field measurements will be taken to assess the precision goal listed in Table 5. The sampling objective is to obtain water quality measurements representative of their location and depth.

Comparability

The multi-parameter water quality sonde used during the water quality sampling will be similar to the instruments used for previous monitoring activities and other monitoring programs. Comparability of the sampling procedures with previous assessments and other

MEP embayments will be achieved through adherence to procedures described in the MEP Benthic Monitoring Field Standard Operating Procedures (Appendix A).

Completeness

Completeness criteria for water quality measurement collection is 100%; all sampling locations specified in Embayment Specific Study Plan must be sampled for water quality to be considered complete. At each station, a hydrographic profile will be taken at the following depths: 0.1 m below the surface, 0.5 m below the surface, every 1 m from depths of 1.0 to 10.0 m, and if the site is deeper than 10 m, every 5 m thereafter. The last set of measurements will be taken at 0.5 m from the bottom. In the event of equipment malfunction, the Chief Scientist will determine the need for appropriate corrective action and will record such action on the Sample Collection and Survey Log forms. A small loss of water quality data (≤10%) over the entire assessment is not expected to compromise the objectives.

B5.1.3 *Grab Sampling*

All sediment samples to be used for faunal analyses will be collected with a 0.04-m^2 Young-modified van Veen grab sampler. A dedicated grab sample, collected by the 0.04-m^2 grab sampler, will provide adequate quantities of sediment for grain size and TOC analysis. Undisturbed samples will be achieved by careful attention to established deployment and recovery procedures, as listed below.

- Thorough wash-down of the grab before each deployment
- Control the depth of sediment penetration by adding or removing weights to the frame and adjusting descent rate
- Slow recovery until grab is free of the bottom
- Inspection for signs of leakage
- Securing the grab on deck

Each grab sample will be inspected for signs of disturbance. The following criteria identify ideal characteristics for an acceptable grab sample:

- Sampler is not overfilled with sediment; the sediment intake is relatively level over the entire area of the grab; and the jaws must be fully closed and the top of the sediment below the level of the opening doors
- Overlying water is present and not excessively turbid
- Sediment depth at the center of the sampler is at least 7 cm, indicating that the desired penetration was achieved

In certain locations, slight overfill may be acceptable at the discretion of the Chief Scientist. Mild overfill may be acceptable according to the following standards:

- The sediment surface is intact

- No evidence that the surface sediment has pushed through the grid surface of the grab, i.e., no visible imprint from the screening outside of that grid
- No evidence that sediment has pushed out through the hinge or the edges of the grab

The Chief Scientist will make the final decision regarding acceptability of all grabs, and the overall condition of the grab will be documented on the station log.

B5.1.3.1 *Benthic Infauna*

Accuracy, Precision, and Representativeness

There will be no subsampling. Consequently, the accuracy, precision, and representativeness of the sampling will depend upon the factors discussed above under Section A7.1.3.

Comparability

Procedures for washing, sieving, and preserving the samples will be consistent with methods described in the MEP Benthic Monitoring Field SOP (Appendix A). Samples will be collected only by trained staff under the supervision of a Chief Scientist with experience in the collection of benthic infaunal samples.

Completeness

All required samples will be collected at all of the stations specified in the Embayment Specific Study Plan. The entire sample will be sieved and all material retained on the 500-µm-mesh screen will be fixed for analysis.

B5.1.3.2 *Sediment*

Accuracy, Precision, and Representativeness

These qualities will be assured by the sampling plan (see B.5.1.2 Grab Sampling above) and by ensuring that samples are well homogenized and subsampled and preserved following methods detailed in Section B2.2.2.

Comparability

Procedures for collecting and preserving the samples will be consistent with methods described in the MEP Benthic Monitoring Field SOP (Appendix A). Procedures for sampling and subsampling are comparable to those used in other investigations in Massachusetts coastal waters.

Completeness

All required samples will be collected at all the stations specified in the Embayment Specific Study Plan.

B5.1.4 *Underwater Digital Images*

The Data Quality Objectives (DQOs) for the field collection of underwater digital images will be met by adhering to the following measures. Still images will be reviewed in the field prior to deploying additional survey equipment or leaving the station to ensure they are of sufficient quality to achieve the objectives of the survey. Real-time viewing of video footage during recording for benthic grab sampling or stand-alone surveys will ensure that the video will be of sufficient quality to achieve the objectives of the survey. High-definition digital still images and video footage will be stored to an external hard drive. All equipment, including the cameras, will be checked thoroughly before each deployment.

Accuracy, Precision, and Representativeness

Accuracy and precision will be ensured by using properly functioning equipment and real-time monitoring of images as described above to acquire analyzable images. Station locations or video transects to be recorded and photographed are those specified in the Embayment Specific Study Plan to elevate embayment benthic habitat quality.

Comparability

The field methods used will be similar to those used for other investigations conducted in Massachusetts coastal waters. All transects and station locations will be occupied so that the nature of the epifauna and sedimentary environment in the area can be compared within an embayment and between years of embayment surveys.

Completeness

All requisite station locations or transects will be recorded on an external hard drive. For digital images collected prior to benthic grabs at least two still images or a minimum of two minutes of video will be recorded at each station. For stand-alone video surveys approximately 20 minutes of high-definition video will be collected at each station. Still images will be reviewed prior to deploying other survey equipment or leave the stations. Video will be monitored in real time during the survey. The external hard drive will be checked in the field to ensure that the images and/or video are recorded.

B5.1.5 *Sediment Profile Imagery (Optional)*

The DQOs for the field collection of the SPI will be met by following several procedures. Proper assembly and operation of the surface video and digital camera SPI system will ensure that images obtained are clear and of high quality. Real-time monitoring of the surface video will permit some degree of evaluation of the potential quality of the deployment. Prior to every field deployment, all video/SPI components are assembled and tested for proper operation. Once the video/SPI system is assembled on board the research vessel, a system check is initiated that includes all features of the system, from tightening all bolts and video cable connectors to testing the video camera and deck video monitor and recorder. Proper system functioning (penetration of prism, flash from digital SPI camera) will be monitored in real time on deck via the video monitor.

Accuracy, Precision, and Representativeness
Accuracy and precision will be ensured by using properly functioning equipment and real-time monitoring of images as described above to acquire clear and analyzable images. Representativeness will be ensured by sampling at all locations specified in the Embayment Specific Study Plan that were chosen to allow for wide geographic coverage.

Comparability
The methods used to collect the sediment profile images will be consistent with those used in other SPI investigations in Massachusetts coastal waters. These methods will be followed consistently by trained staff members throughout the program.

Completeness
To ensure that all required images are collected at all planned stations, the digital image counter will be checked to confirm that the system was functioning properly after every station. Any misfires or improper camera operation will be corrected while on station. Almost any electronic or mechanical failure of the profile camera can be repaired in the field. Spare parts and a complete back-up camera will be carried on each SPI survey.

B5.1.6 *Hard-bottom/Riprap Destructive Samples (Optional)*

Accuracy, Precision, and Representativeness
There will be no subsampling. Consequently, the accuracy, precision, and representativeness of the sampling will depend upon the factors discussed above under Section A7.1.6.

Comparability
Procedures for collecting and preserving the samples will be consistent with methods described in the MEP Benthic Monitoring Field SOP (Appendix A). Samples will be collected only by trained staff under the supervision of a Chief Scientist with experience in the collection of hard-bottom destructive samples.

Completeness
All required samples will be collected at all the stations specified in the Embayment Specific Study Plan.

B5.2 Laboratory Activities

B5.2.1 *Macrofauna Analysis*

Accuracy
Benthic macrofauna will be identified by experienced taxonomists at a contracted laboratory. In cases where different taxonomists identify replicates from the same station, discrepancies in species identifications will be recognized during data entry and reviewed. Taxonomic discrepancies will be addressed by communication among the taxonomists. In the case of questions about organisms in specific taxonomic groups, specimens may be sent to recognized

experts for a second opinion on the identification. Standard taxonomic references will be used, and selected specimens of newly found species will be retained as part of the reference collection.

Precision

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 ≤ 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 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 (Appendix B).

Identification and Enumeration: The same basic QC principles described in the Sorting section will in general apply to species identification. 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 (Appendix B).

Representativeness

Because all the samples will be analyzed, representativeness will be determined by sampling factors.

Completeness

Since one sample from each station will be archived, the loss of one sample will still permit data to be obtained from the archived sample for that station. One hundred percent completeness is expected.

Comparability

Methods of analysis will be comparable to those used in other investigations conducted in Massachusetts coastal waters. Comparability of the identifications will be ensured through the use of standard taxonomic references and by comparison of specimens in the MEP Benthic Monitoring Reference Collection. Taxonomists will be familiar with fauna from Massachusetts waters and those of the surrounding regions. The reference collection will be maintained and, if new species are identified, expanded. Any new species that have not been reported in previous MEP Benthic Monitoring surveys or other studies conducted in the Massachusetts coastal waters will be checked against similar taxa in the reference collection and carefully verified with recognized experts.

B5.2.2 *Underwater Digital Image Analysis*

Accuracy and Precision

High-definition still images and video footage 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 because of visual resolution and time constraints. Encrusting, cryptic, or very abundant taxa will not be counted from the still images due to time constraints. To control for analyst error, 10% of images will be reanalyzed by a different reviewer to assess variability.

Completeness

All appropriate still images and video footage will be analyzed according to specification in the Embayment Specific Study Plan.

Comparability

The methods of collection and analysis of the still images and video footage will be similar between surveys and to other studies conducted in Massachusetts coastal waters to allow comparisons between data collected from different survey years. The method of analysis for the still images and video footage will be similar enough to permit qualitative comparisons.

Representativeness

Biological assemblages are routinely documented using still images and video footage. The location of the photographic coverage is usually constrained by (1) mobility of the ship during station occupation or transect transit due to surface currents and wind, and (2) bottom visibility (moving in a down-current direction frequently causes reduced visibility due to sediment clouds). Within these constraints, representative still images and/or visual footage of each area will be obtained.

Due to the 3-dimensional nature of the video footage, qualitative characterization of habitat relief and habitat and biotic heterogeneity is usually easier from the video footage. Additionally, the video footage covers more area and is thus used to document the occurrence of larger, more sparsely distributed fauna.

B5.2.3 *Physicochemical Parameters*

Sediment samples collected will be analyzed for sediment grain size and TOC by a contracted laboratory as described in the MEP Benthic Monitoring Laboratory SOP (Appendix B). The laboratory will follow all QC/QA procedures for the analytical method being followed. No equipment and field blanks for sediment chemistry are required.

To estimate the overall precision or repeatability of results, a subset of field samples are replicated by taking co-located (a new location within 37m radius around the site) and sequential duplicate samples. At a minimum 10% of the total number of samples group is typically collected as a duplicate. For this project a minimum of 10% of grain size and TOC sediment samples will be collected as a duplicate. Adequate sediment is collected for the analytical laboratories to perform the required matrix spike/matrix spike duplicate (MS/MSD) analyses.

B5.2.4 *Sediment Profile Image Analysis (Optional)*

Accuracy

Control of the computer image analysis includes system preparation, actual image analysis, and data reduction. A set of standard instructions is followed in setting up the image processor. Once the system is on and functioning, a standardized scale slide is measured to ensure that the linear measurements made on the profile images are accurate.

Precision

Even with the most careful control, there may be variations in external lighting that cause subtle color differences among images. 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.

Completeness
The three best images taken at each station, if usable, will be analyzed.

Comparability
The comparability of the SPI analyses will be ensured by consistent application of QC procedures and by using the same analyst throughout the project whenever possible. The analyses will be comparable to similar analysis performed for other studies in Massachusetts coastal waters. However, 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.

Representativeness
Representativeness is defined by the stations selected in the baseline.

B6. INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE

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

B6.1 Laboratory Equipment

Microscopes used for sorting of faunal samples and taxonomic identification of specimens are cleaned and maintained as needed.

No analytical laboratory instruments are covered by this QAPP.

B6.2 Underwater Digital Images

All the maintenance and calibrations of the digital cameras will be carried out prior to the survey, in accordance with the manufacturer's specifications, and are the responsibility of the entity conducting the survey.

B6.3 Sediment Profile Image Analysis System (Optional)

Prior to every field deployment, all video components are collected and tested for proper operation. Once the video SPI system is assembled on board the research vessel, a system check is initiated. This check includes all features of the video SPI system, from tightening all bolts and video cable connectors to testing the video camera and deck video monitor and recorder. In addition, before every field deployment, the clock in the SPI system will be set to match the clock used by the navigation system aboard the research vessel.

Proper system functioning (e.g., penetration of prism, flash from digital SPI camera) will be monitored in real time on deck via the video monitor. Any misfires or improper camera operation can then be corrected while on station. Almost any electronic or mechanical failure of the video camera can be repaired in the field. Spare parts and complete back-up video and digital cameras will be brought to each survey.

B6.4 Hard-bottom/Riprap Destructive Sampling (Optional)

Prior to every field deployment, all air dredge equipment will be inspected and tested for proper operation. Any maintenance or repairs to the equipment will be done prior to arrival at the research vessel.

No scuba diving equipment is covered by this QAPP.

B7. INSTRUMENT/EQUIPMENT CALIBRATION AND FREQUENCY

B7.1 Navigation Equipment

GPS units on the research vessels will be maintained and calibrated to the manufacturer's specifications.

B7.2 Multi-Parameter Water Quality Sonde

Test and calibrate the multi-parameter sonde following the manufacturer's calibration and maintenance procedures. Once each week, verify that the sonde is functioning properly by performing manufacturer recommended internal diagnostic readouts (e.g., pH millivolts, cell constants, and/or other diagnostic readings). Records of these checks should be saved in a Calibration Log or other documentation. Water quality sondes will be calibrated at the start and end of each sampling day following the manufacturer's recommended procedures for each probe. A post-calibration measurement check will also be recorded (checked against the same standard solutions used for calibration) before any re-calibration to provide a measure of instrument "accuracy" in the field and/or drift. The calibrations and any checks will be recorded on a Calibration Log or a manufacturer-provided calibration worksheet.

B7.3 Laboratory Equipment

No analytical laboratory instruments are covered by this QAPP.

B8. INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES

Critical supplies for field activities will be the responsibility of the Chief Scientist (Table 19).

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

Table 19. Supplies, Acceptance Criteria, and Responsibility for Critical Field Supplies.

Critical Supplies and Consumables	Inspection Requirements and Acceptance Criteria	Responsible Individual
Jars for macrofaunal samples	Visually inspected for cracks, breakage, and cleanliness. May be reused.	Chief Scientist
Sample bottles for sediment chemistry	Visually inspected upon receipt for cracks, breakage, and cleanliness. Must be accompanied by certificate of analysis.	Chief Scientist
Chemicals and reagents	Visually inspected for proper labeling, expiration dates, appropriate grade.	Chief Scientist Laboratory Staff
Sampling equipment (grabs)	Visually inspected for obvious defects, damage, and contamination.	Chief Scientist
SPI camera system	Visually inspected for obvious defects or damage; electronics tested.	Vendor Chief Scientist
HD digital cameras	Visually inspected for obvious defects or damage; electronics tested	Chief Scientist
Navigation instruments	Functional checks to ensure proper calibration and operating capacity.	Vessel Captain Chief Scientist

B9. NON-DIRECT MEASUREMENTS

Non-direct data (historical reports, maps, literature searches, and previously collected analytical data) may be used in the preparation of the Embayment Specific Study Plan (Task 1). These data may come from sources such as:

- Prior MEP embayment reports
- Results of other MassDEP studies including water quality monitoring and eelgrass survey data
- Pertinent data collected by other agencies, such as USGS bathymetry data and NOAA weather records, as appropriate
- Peer-reviewed publications of surveys completed in the embayment or embayment system of interest

B10. DATA MANAGEMENT

B10.1 Data Custody

Custody of field data will be the responsibility of the Chief Scientist during the field activity. Field data will be recorded electronically or manually on the field datasheets. Electronic water quality data will be provided to the Project Manager within one week of collection.

Laboratory managers will be responsible for custody of data generated by contracted laboratories (see below).

Each team member involved in this project is responsible for the internal custody of their electronic and hard-copy data until they are submitted to the Project Manager. All hand-entered data that are submitted electronically will receive 100% verification prior to submission, will be entered and checked using double data entry, or will be completed using a software application (e.g., KeyesPunch™), which employs automated controls and data verification. Formats designed to comply with rules of the selected database will be used in the application to constrain data entry. These features will ensure that any entry errors are caught and corrected as the operator keys the data.

B10.2 Laboratory Data and Data Reduction

All data generated by contracted laboratories will be either electronically transferred from the instrument or manually read from the instrument display (optical field of a microscope or video monitor) and entered directly into an electronic format (e.g., Excel spreadsheet in the case of SPI data and digital image data), 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.

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 and SPI data analysis discussed below require 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 Section D2.

The format for final data submission is described below.

B10.2.1 *Macrofaunal Analysis*

The contracted laboratory will include the scientific name for each taxon in the macrofaunal abundance data submitted to the Project Manager. Taxonomic consistency between the current and previous survey identifications will be verified using the project reference collection (see Sections B4.1, B4.4, and B5.2.2).

Macrofaunal data will be analyzed for the following community parameters: abundance, Shannon-Wiener diversity index (H'), Pielou's evenness (J'), Margalef's diversity index, Simpson, and Average Taxonomic Distinctiveness (AvTD; using the Primer software package).

Shannon-Wiener diversity index (H') characterizes the species diversity in a community and is calculated by

$$H' = - \sum_{i=1}^{R} p_i \ln p_i \qquad (1)$$

where p_i is the proportion of individuals belonging to the *i*th species in the sample.

Pielou's evenness is calculated by

$$J' = H'/H'_{max} \quad (2)$$

where H' is derived from the Shannon-Wiener diversity index and H'_{max} is the maximum possible value of H' (if every species was equally likely), calculated by H' = ln *S*. *S* is species richness, the total number of species in the sample. J' ranges between 0 and 1, the lower J' is the less evenness is a community between the species.

Margalef's diversity index (D_{Mg}) is calculated by

$$D_{Mg} = (S - 1)/\ln N \quad (3)$$

where *S* is species richness and N is the total number of individuals in the sample.

Multivariate analyses will be performed using a statistical software package (e.g., R, PRIMER) to determine the spatial patterns in the overall similarity of benthic assemblages in the survey area. Multivariate analyses will include Bray-Curtis similarity hierarchical agglomerative clustering (cluster analysis) and non-metric multidimensional scaling (nMDS; Clarke 1993, Warwick 1993, Clarke and Green 1988). A fourth root transformation will be used on infaunal abundance data to ensure that all taxa, not just the numerical dominants, will contribute to similarity measures. The "similarity profile test" (SIMPROF) will be used to provide statistical support for the identification of faunal assemblages (i.e., selection of cluster groups). SIMPROF is a permutation test of the null hypothesis that the groups identified by cluster analysis do not differ from each other in multivariate structure.

US M-AMBI, a multivariate AZTI Marine Biotic Index (M-AMBI) adapted for US coastal waters, will be used to determine the health condition of sub-embayments and embayment. US M-AMBI will be calculated following Pelletier et al. (2018); however, prior to the calculation of US M-AMBI, the laboratory or researcher may need to contact the first author of Pelletier et al. (2018), Marguerite C. Pelletier[3], regarding any updates in coding and/or Environmental Grouping (EG) assignment. US M-AMBI health condition categories will include: bad (<0.20), poor (0.20 to 0.39), moderate (0.39 to 0.53), good (0.53 to 0.77), and high (>0.77). A statistical routines package (e.g., R, PRIMER) will be used to calculate this index.

The results for abundance, H' diversity, J' evenness, Margalef's diversity index, Simpson, AvTD, and US M-AMBI will be combined and tabulated into an Excel spreadsheet for delivery to the Project Manager. Hierarchical agglomerative clustering (cluster analysis) and nMDS multivariate analyses will be provided in graphic format to the Project Manager.

[3] Marquerite Pelletier: Ph.D. at US EPA, Atlantic Coastal Environmental Sciences Division (ACESD) Laboratory, Narragansett, RI.

B10.2.2 *Sediment Analysis*

The contracted laboratory will include sediment grain size and percentage of total organic carbon in the sediment data submitted to the Project Manager. After data verification sediment samples can be disposed of following internal laboratory protocols. Sediment samples with known toxins (e.g., PCBs, dioxin, and PAHs) will be disposed of properly following local, state, and federal laws.

B10.2.3 *Underwater Digital Image Analysis*

There is no manipulation of underwater digital image data prior to submission.

B10.2.4 *SPI Analysis (Optional)*

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 for delivery to the Project Manager.

SPI data are used to summarize environmental conditions through the calculation of the Organism-Sediment Index (OSI). The OSI (Rhoads and Germano 1986) is an integrative estimate of the general ability of the benthic habitat to support fauna. The OSI is defined from SPI parameters and the indirect estimation of bottom dissolved oxygen levels. The lowest value of the OSI (-10) denotes habitats that have little or no dissolved oxygen, no apparent evidence of surface or subsurface fauna, and where methane gas is present (subsurface data). The highest value of the OSI (+11) is given to habitats that have high dissolved oxygen, a deep apparent RPD layer, evidence of fauna, and no methane gas. The index is calculated by using the RPD depth, the successional stage, the presence of methane voids, and visual indications of low oxygen concentrations in the water column. The formulation for the OSI is shown in Table 20.

B10.3 Dataset Structure

Electronic Data Deliverables will be prepared by the contracted laboratory in a structure and format that complies with the selected database.

B10.4 Project Database Codes

Standardized codes and qualifiers help to ensure consistency over time in a benthic monitoring program. The internal sample data codes that may be used for sampling events, sampling locations, benthic samples, and grab and hard bottom/riprap destructive sample bottles are shown in Table 21 through 24, respectively. The data codes that may be used with water quality data are shown in Table 25. The data codes that may be used with the benthic macrofaunal (i.e., benthic infaunal and hard-bottom destructive) and sediment samples are presented in Table 26. Benthic macrofaunal taxonomic identification and benthic community parameters data codes are shown in Tables 27 and 28. Tables 29, 30, and 31 show the parameters codes for sediment analysis, sediment grain size and TOC analysis, and sediment grain size result codes, respectively. The underwater digital image survey and analysis codes

are listed in Tables 32 and 33. Tables 34 and 35 contain the database codes that are applicable only to the optional SPI survey and data analysis. Additional database codes that may be used for the benthic monitoring tasks (Tasks 2 and 3) are included in Table 36. These database codes are generally consistent with the NCCA program.

New codes must be requested before the data are submitted. Additional codes may be added to the MEP Benthic Monitoring by the MassDEP MEP Program Manager when requested by the Project Manager. New codes that have been requested by Project Managers will be included in future revisions of the MEP Benthic Monitoring QAPP.

Table 20. Formulation of the Organism-Sediment Index (OSI).

SPI Parameter	Score	Hypothetical Example
		Station A
RPD Depth (cm) (choose one value)		
0	0	
>0-0.75	1	
0.76-1.50	2	X
1.51-2.25	3	
2.26-3.00	4	
3.01-3.75	5	
>3.75	6	
Successional Stage (choose one value)		
Azoic	-4	
Stage I	1	X
Stage I-II	2	
Stage II	3	
Stage II-III	4	
Stage III	5	
Stage I on III	5	
Stage II on III	5	
Sediment/Near-bottom Gas (choose neither, one, or both as appropriate)		
Methane	-2	
No/Low DO	-4	X
	Calculated OSI	-1

Table 21. Data codes for sampling events (All sampling methods).

Field	Format	Description
EVENT_ID	Alphanumeric	Identifier of sampling event (survey)
EVENT_NAME	Alphanumeric	Name of the event
PLAT_NAME	Alphanumeric	Platform name (e.g., vessel name).
CHIEF_SCIENTIST	Character	Name of the scientist in charge of the event
COMMENTS	Character	Comments on survey event, detailing any exceptions from standard procedures

Table 22. Data codes for benthic sampling locations (stations; all sampling methods).

Field	Format	Description
EVENT_ID	Alphanumeric	Identifier of sampling event (survey)
STAT_ID	Alphanumeric	Identifier for station
STAT_ARRIV_LOCAL	MM/DD/YY HH:MM	Station arrival date and time (local time)
BEG_LATITUDE	Numeric	Beginning latitude measured at each station visit (decimal degrees)
BEG_LONGITUDE	Numeric	Beginning longitude measured at each station visit (decimal degrees)
END_LATITUDE	Numeric	Ending latitude measured at each station visit (decimal degrees)
END_LONGITUDE	Numeric	Ending longitude measured at each station visit (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 detailing any exceptions from standard procedures on this station visit

Table 23. Data codes for benthic samples (All sampling methods).

Field	Format	Description
EVENT_ID	Alphanumeric	Identifier of sampling event (survey)
STAT_ID	Alphanumeric	Identifier for station
STAT_ARRIV_LOCAL	MM/DD/YY HH:MM	Station arrival date and time (local time)
SAMPLE_ID	Alphanumeric	Sample identifier
GEAR_CODE	Character	Code for type of gear used to collect sample
GRAB_DEPTH	Numeric	Depth of sediment sample, from sediment surface to bottom of sample, in cm
SAMPLE_DATE_TIME_LOCAL	MM/DD/YY HH:MM	Date and time sample was taken (local time)
LATITUDE	Numeric	Precise latitude recorded when sample was collected.
LONGITUDE	Numeric	Precise longitude recorded when sample was collected.
SAMP_VOL	Numeric	Volume of sample as collected, only for grab samples
SAMP_VOL_UNIT_CODE	Character	Unit of volume measurement, only for grab samples
COMMENTS	Alphanumeric	Comments for this sample
LOC_COMMENTS	Alphanumeric	Comments for the exact sample location

Table 24. Bottle data codes for benthic grab (infaunal and sediment samples) and hard bottom/riprap destructive samples.

Field	Format	Description
EVENT_ID	Alphanumeric	Identifier of sampling event (survey)
SAMPLE_ID	Alphanumeric	Sample identifier
BOTTLE_ID	Alphanumeric	Subsample (bottle) identifier
COMMENTS	Alphanumeric	Comments for a given bottle

Table 25. Water quality data codes.

Field	Format	Description	
EVENT_ID	Character	Identifier of sampling event (survey)	
DATE COLLECTED	MMDDYY	Date sample was taken	
STAT_ID	Character	Station identification code as used on sample label	
DEPTH	Numeric	Depth that the water quality data was collected at (m)	
TEMP	Numeric	Water temperature	
DO	Numeric	Dissolved oxygen (mg/L)	
PH	Numeric	pH	
SALINITY	Numeric	Salinity (ppm)	
QA FLAG (if appropriate)	Character	QA/QC flag (additional flags maybe used, if defined in QA_COMMENTS field)	
		Flag	Definition
		P	Probe malfunction (explain in QA_COMMENTS field)
		Q	Other quality concerns, not identified above
QA_COMMENTS	Character	Explanation for Q FLAG (if needed)	

Table 26. Benthic macrofaunal and destructive sample data codes. Data codes are revised from NCCA 2020 (US EPA 2021b).

Field	Format	Description	
EVENT_ID	Character	Identifier of sampling event (survey)	
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_ID	Character	Sample ID as used on field sheet (on sample label)	
SAMPLE_TYPE	Character	INFAUNAL or DESTRUCTIVE	
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 27. Benthic macrofaunal taxonomic identification data codes. Data codes are revised from NCCA 2020 (US EPA 2021b).

Field	Format	Description	
EVENT_ID	Character	Identifier of sampling event (survey)	
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_ID	Character	Sample ID 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	
APHIA_ID	Numeric	World Register of Marine Resources (WoRMS) Database ID. 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 WoRMS.	
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
		IM	Immature
		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

Table 27. Continued.

Field	Format	Description
COND_COMMENTS	Character	Explanation for Q FLAG (if needed)
LAB_COMMENTS	Character	General laboratory analysis comments

Table 28. Benthic community parameter data codes.

Field	Format	Description
YEAR	Numeric	Survey year
EVENT_ID	Character	Identifier of sampling event (survey)
SITE_ID	Character	Site identification code as used on sample label
SAMPLE_ID	Character	Sample ID as used on chain of custody form (on sample label)
SPECIES_COUNT	Numeric	The number of species in a sample.
NUMBER_IND_M2	Numeric	Total number of individuals per square meter of bottom
D_RICHNESS	Numeric	Margalef's diversity index value
H'_DIVERSITY	Numeric	Shannon-Wiener diversity index value
J_EVENNESS	Numeric	Pielou's evenness index value
1-λ_SIMPSON	Numeric	Simpson diversity index value
DELTA_+	Numeric	Average taxonomic distinctness value
BI	Numeric	Biological Index
US_M_AMBI	Numeric	Multivariate AZTI Marine Biotic Index in US Coastal Waters
CATEGORY	Character	US M AMBI health condition category

Table 29. Sediment analysis data codes. Data codes revised from NCCA 2020 (US EPA 2021b).

Field	Format	Description	
EVENT_ID	Character	Identifier of sampling event (survey)	
STAT_ID	Character	Station identification code as used on sample label	
SAMPLE_ID	Character	Sample ID as used on chain of custody form (on sample label)	
DATE_COLLECTED	MMDDYY	Date sample that the field crew collected the sample	
LAB_NAME	Character	Name of lab	
DATE_RECEIVED	MM/DD/YY	Date sample was received by lab	
ANALYSIS_TYPE	Numeric	GRAIN SIZE, TOC, or Contaminant	
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)	

Table 30. Analysis data codes for TOC and other parameters including contaminants with single result values. Data codes are revised from NCCA 2020 (US EPA 2021b).

Field	Format	Description	
EVENT_ID	Character	Identifier of sampling event (survey)	
STAT_ID	Character	Station identification code as used on sample label	
SAMPLE_ID	Character	Sample ID as used on chain of custody form (on sample label)	
SAMPLE_NUMBER	Character	Sample number as used on chain of custody form (on sample	
DATE_COLLECTED	MMDDYY	Date sample that the field crew collected the sample	
LAB_NAME	Character	Name of lab	
DATE_RECEIVED	MM/DD/YY	Date sample was received by lab	
ANALYSIS_TYPE	Character	TOC or Contaminant	
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_ANALYZED	MMDDYY	Date that the analysis started	
HOLDING_TIME	Y/N performed within holding time	Analysis performed within holding time	
MDL	Numeric	Lab method detection limit	
RL	Numeric	Actual Reporting limit (based on the unique matrix of sediment for each batch of samples)	
MOISTURE	Numeric	Moisture in the sample (value used by lab to convert wet units to dry)	
MOIST_UNIT	Character	Unit used to report moisture (% or mg/kg)	

Table 30. Continued.

Field	Format	Description
DILUTION	Numeric	Dilution of sample (blank if no dilution)
RESULT	Numeric	Concentration value
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 of the analysis

Table 31. Sediment grain size analysis data codes. Data codes are revised from NCCA 2020 (US EPA 2021b).

Field	Format	Description	
YEAR	Numeric	Survey year	
EVENT_ID	Character	Identifier of sampling event (survey)	
STAT_ID	Character	Station identification code	
SAMPLE_ID	Character	Sample ID as used on chain of custody form (on sample label)	
SAMPLE_NUMBER	Character	Sample number as used on chain of custody form (on sample label)	
DATE_COLLECTED	MM/DD/YY HH:MM	Date sample that the field crew collected the sample	
ANALYSIS_TYPE	Numeric	GRAIN SIZE	
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	

Table 31. Continued.

Field	Format	Description
METHOD	Character	Laboratory method used
DATE_PROCESSED	MM/DD/YY	Date that the analysis started
HOLDING_TIME	Y/N	Analysis performed within holding time
MDL	Numeric	Lab method detection limit
RL	Numeric	Actual Reporting limit (based on the unique matrix of sediment for each batch of samples)
MOISTURE	Numeric	Moisture in the sample (value used by lab to convert wet units to dry)
MOIST_UNIT	Character	Unit used to report moisture (% or mg/kg)
DILUTION	Numeric	Dilution of sample (blank if no dilution)
PCT_Gravel	Numeric	Percentage of gravel
PCT_VC_Sand	Numeric	Percentage of very coarse sand
PCT_C_Sand	Numeric	Percentage of coarse sand
PCT_M_Sand	Numeric	Percentage of medium sand
PCT_F_Sand	Numeric	Percentage of fine sand
PCT_VF_Sand	Numeric	Percentage of very fine sand
PCT_Silt	Numeric	Percentage of silt
PCT_Clay	Numeric	Percentage of clay
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 of the analysis

Table 32. Underwater digital image survey codes.

Field	Format	Description
EVENT_ID	Character	Identifier of sampling event (survey)
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	MM/DD/YY HH:MM	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 33. Underwater digital image analysis data codes.

Field	Format	Description	
EVENT_ID	Character	Identifier of sampling event (survey)	
LAB_NAME	Character	Name of lab	
SITE_ID	Character	Site identification code	
DATE_COLLECTED	MMDDYY	Date image was taken	
STAT_ARRIV_LOCAL	MM/DD/YY HH:MM	Station arrival date and time (local time)	
IMAGE_DATE_TIME_BEG_LOCAL	MM/DD/YY HH:MM	Video only: Time of the beginning of this video (local time)	
IMAGE_DATE_TIME_END_LOCAL	MM/DD/YY HH:MM	Video only: Time of the end of this video (local time)	
USABLE_MINUTES	Numeric	Video only: Number of usable minutes between the image_date_time_beg and image_date_time_end	
DEPTH_BEG	Numeric	Video only: Depth of water at image_time_beg (meters)	
DEPTH_END	Numeric	Video only: Depth of water in at image_time_end (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
		rr	Riprap
		s	Sand
RELIEF_CODE	Character	Codes describing the habitat relief observed in the digital image	
		Code	Definition (See Table 14)
		n	None
		l	Low
		m	Moderate
		h	High

Table 33. Continued.

Field	Format	Description	
SED_DRAPE_CODE	Character	Codes describing the sediment drape observed in the digital image	
		Code	Definition (See Table 15)
		a	Absent
		l	Low
		m	Moderate
		h	High
SUSP_MATTER_CODE	Character	Codes describing the suspended matter observed in the digital image	
		Code	Definition (See Table 15)
		a	Absent
		l	Low
		m	Moderate
		h	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 16 and 17)
		a	Absent
		r	Rare
		p	Present
		c	Common
		ab	Abundant
ANAL_COMMENTS	Character	General laboratory analysis comments	

Table 34. Sediment profile imaging survey data codes.

Field	Format	Description
EVENT_ID	Character	Identifier of sampling event (survey)
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	MM/DD/YY HH:MM	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 35. SPI analysis data codes.

Field	Format	Unit	Description
EVENT_ID	Character		Identifier of sampling event (survey)
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

Table 36. Descriptions of Other Database Codes used in the MEP Benthic Monitoring Program.

Field	Format	Description	
DEPTH_UNIT	Numeric	Codes describing the depth of a station	
		Code	Definition
		m	Meters
		cm	Centimeters
GEAR_CODE	Character	Codes describing the sampling gear	
		Code	Definition
		AIRLIFT	Airlift dredge and a scraper
		DIGI_CAM	High-definition digital camera
		SPI_DIGI	Sediment Profile Camera System with Digital Camera
		VV04	0.04-m^2 Young-modified Van Veen Grab
INSTR_CODE	Character	Codes describing the instrument used	
		Code	Definition
		MICR	Microscope
		RULER	Measurement by ruler
MATRIX_CODE	Character	SED	Sediment
METH_CODE	Character	ENUM	Enumeration
UNIT_CODE	Character	Codes describing units	
		Code	Definition
		m	Meters
		cm	Centimeters
		m3	Cubic meter
VAL_QUAL	Character	Codes describing value quality	
		Code	Definition
		A	Value above maximum detection limit, *e.g.*, too numerous to count or beyond range of instrument. DETECT_LIMIT is the maximum detection limit or maximum penetration depth for SPI RPD measurements.
		F	Abundance recorded for a fraction or portion of the sample collected
		P	Present but uncountable, value given is NULL
		ND	Usable non-detect result; not detected at or above the method detection limit (MDL). Database value input as null or negative. DETECT_LIMIT is the MDL.
		d	Accuracy does not meet data quality objectives.

Table 36. Continued.

Field	Format	Description	
		e	Results not reported, value given is NULL. Explanation in COMMENTS field
		ML	Sample label is missing.
		I	Possibly suspect/invalid and not fit for use. Investigation pending.
		r	Precision does not meet data quality objectives.
		S	Suspect/Invalid. Not fit for use. Explanation in COMMENTS field
SPEC_QUAL	Character	Codes describing species quality	
		Code	Definition
		G	Fragment
		J	Juvenile (unspecified stage)
		X	Complex

B10.5 Data Submittal to MassDEP and Selected Database

Prior to submittal to MassDEP and the selected database, all data will receive a quality assurance review by the contracted laboratory and the Project Manager during which a software application will be used for logical error checks and to check for violations of database constraints and business rules. Any issues will be corrected in the data files. Any irresolvable issues in the data files identified by quality control checks (for example, stations more than specified distance from target) will be mentioned in the data deliverable to MassDEP.

Electronic data submissions will be made by the Project Manager or a designee using email or a secure File Transfer Protocol (FTP) site to MassDEP. Submissions by the Project Manager or a designee to the selected database will be made through the database interface.

The data deliverables that will be provided to MassDEP in the data code format described in Tables 33 through 36 or image formats (e.g., jpeg, tiff, gif, png) are presented in Table 37.

Table 37. Data deliverables that will be provided to MassDEP.

Task	Data Deliverable
Task 2: Benthic Monitoring Surveys	• Latitude and longitude coordinates of each sampling location • Water quality profile measurements • Digital still images taken prior to grab sampling • Digital video (if conducted) • Hard bottom/riprap images (If conducted)
Task 3: Analysis of Benthic Surveys	• Sediment grain size and TOC results • Benthic infaunal taxa list by station • Calculated benthic infaunal indices • SPI parameters (if conducted) • Analyzed SPI images (If conducted) • Macrofaunal hard bottom/riprap taxa list by station (if conducted) • Macrofaunal hard bottom/riprap calculated indices (if conducted) • Digital still or video parameters (if images are analyzed)

C. ASSESSMENT AND OVERSIGHT

C1. ASSESSMENT AND RESPONSE ACTIONS

This section identifies the number, frequency, and type of planned assessment activities that will be performed to assure implementation of this QAPP for MEP benthic monitoring. These activities will be overseen by the contracted laboratory QA Officer.

C1.1 Assessments

C1.1.1 *Field Sampling Readiness Reviews*

Each Embayment Specific Study Plan will include a section containing a field survey plan (Section A9.4.2). This section will reference the specific field activities to be conducted and the sections of the MEP Benthic Monitoring Field SOP to be followed. The Field SOP includes required equipment lists. An example is shown in Table 38.

C1.1.2 *Field Sampling Technical System Audit*

The QA Manager will be responsible for periodic internal Technical Surveillance Audits (TSAs) to verify that field sampling procedures and measurements are properly followed. The internal field audit checklist (Table 39) will include examination of the following:

- Field sampling records
- Sample collection, handling, and packaging procedures
- Adherence to the Field SOP and this QAPP
- QA procedures
- Chain-of-custody
- Sample documentation

Results of internal field TSAs will be documented in the QA reports to the Program Manager. (Section C2).

C1.1.3 *Laboratory Technical System Audits*

System audits are performed as described in each laboratory's QA manual for internal auditing. Laboratory audits may be conducted by the contracted laboratory's QC/QA Manager at the project start up and then periodically as part of its analytical monitoring program. The laboratory audit checklist (Table 40) will review the following:

- QA organization and procedures
- Personnel training and qualifications
- Sample log-in procedures
- Sample storage facilities
- Analyst technique
- Adherence to laboratory SOPs and this QAPP
- Compliance with QA/QC objectives

- Instrument calibration and maintenance
- Facility security
- Waste management
- Data recording, reduction, review, reports, and archival
- Cleanliness and housekeeping

Preliminary results of the systems audit will be discussed with the Laboratory management staff. A written report that summarizes audit findings and recommends corrective actions will be prepared and submitted to the Laboratory Director for response and to the Program Manager. The results of the audit, including resolution of any deficiencies, will be included in the QA reports, as described in Section C2.

C1.1.4 *Performance Evaluation Sample Assessment*

Proficiency testing for infaunal taxonomic analyses is accomplished through regular communication and inter-calibration of infaunal samples among taxonomists.

C1.1.5 *Data Technical System Audits*

Data will be audited under the direction of the QA Manager as part of the data validation process (Section D.1). Raw data will be reviewed for completeness and proper documentation. Errors noted in data audits will be communicated to analysts and laboratory management and corrected data will be verified. Audits of the data collection procedures at contracted laboratories will be the responsibility of the contracted laboratories. Each contracted laboratory is fully responsible for the verification and validation of the data it submits. Data must be submitted in QAPP-prescribed formats; no other formats will be acceptable. During the time that work is in progress, the contracted laboratory's QA Officer or his/her designee will conduct an inspection to evaluate the laboratory data-production process. All data must be reviewed by the contracted laboratories QA Officer or designee prior to submission to the Project Manager.

C1.2 Assessment Findings and Corrective Action Responses

All technical personnel share responsibility for identifying and resolving problems encountered in the routine performance of their duties. Issues that affect the schedule, cost, or performance of project tasks will be reported to the Project Manager. The Project Manager will be accountable for overall conduct of the MEP Benthic Monitoring Project for a specific embayment, including the schedule, costs, and technical performance. The Project Manager will be responsible for identifying and resolving problems that (1) have not been addressed in a timely manner or successfully at a lower level, (2) influence multiple components of the project, or (3) require consultation with contracted laboratories or MassDEP. He/she will be responsible for evaluating the overall impact of the problem on the project and for discussing corrective actions with the Town(s) and MassDEP MEP Program Manager. The Project Manager will also identify and resolve problems that necessitate changes to this QAPP. Problems identified by the QA Manger will be reported to the Project Manager and corrected as described in Section C2.

Table 38. Equipment list in the Field Standard Operating Procedures for Soft-Bottom Infaunal and Sediment Sampling.

A 0.04-m^2 Ted Young-modified Van Veen grab sampler will be used to collect soft-bottom sediment samples for benthic macrofauna and sediment characteristics (grain size and TOC) analyses. Following is the supply list for collecting samples:

- Young-modified Van Veen grab with grab stand or frame if needed
- Weights and pads for grab
- Nitrile gloves
- Plastic tub or bucket
- 0.500 mm screening bucket or stainless steel sieve
- Sieve box or bucket
- Electrical tape
- Forceps (fine-tipped)
- Funnel (wide-mouth)
- Formalin[4] (37% formaldehyde)
- Borax[5]
- Rose Bengal stain
- Ruler (cm)
- Squirt bottle (ambient water)
- Stainless steel mixing pot or bowl with lid
- Stainless steel or Teflon spoons (15”), scoops, or spatula
- Glass, Nalgene or other sturdy plastic, wide-mouth sample jars (500 ml) with lids
- Glass, Nalgene or other sturdy plastic, wide-mouth sample jars (125 ml) with lids
- Plastic bags (e.g., Whirl Pak, for grain size samples)
- Scrub brush
- Soap (biodegradable)
- Cooler with wet ice.

The following items will also be needed for recording measurements:

- Survey Log form
- Sample Collection form
- #2 Pencils
- Write-on yellow tape or pre-printed labels
- Clear tape strips
- Fine-tipped indelible markers
- Waterproof paper for internal sample jar labels

[4] Follow safe chemical handling procedures including the use of personal protective equipment (e.g., gloves and safety glasses), the manufacture’s Safety Data Sheet recommendations, and the OSHA Formaldehyde Standard (29 CFR 1910, 1048) when using this chemical.

[5] A prepared 10% buffered formalin solution can be purchased through laboratory chemical suppliers.

Table 39. Example of Internal Field Technical Surveillance Audit Checklist.

Project: **Site Location:** **Auditor:**	
1. Was project-specific training held?	
2. Are copies of the MEP Benthic Monitoring QAPP, Field SOP, and Embayment Specific Study Plan on site and available to personnel?	
3. Are samples being collected in accordance with the Study Plan and Field SOP?	
4. Do the numbers and locations of samples conform to the Study Plan?	
5. Are samples labeled in accordance with the Study Plan?	
6. Is field instrumentation being operated and calibrated in accordance with the QAPP and Field SOP?	
7. Are samples being preserved and containerized in accordance with the QAPP and Field SOP?	
8. Are chain-of-custody procedures and documents in conformance with the QAPP and Field SOP?	
9. Are field records complete, accurate, up-to-date, and in conformance to good recordkeeping procedures?	
10. Are modifications to the QAPP, Field SOP, and/or Study Plan being communicated, approved, and documented appropriately?	
Additional Comments:	
Auditor:	**Date:**

Table 40. Example of a Laboratory Technical Systems Audit Checklist.

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

Corrective actions may result from planned audits or from unanticipated events that occur during the course of the project. Significant events that result in deviations from this QAPP will be recorded through the "Extraordinary Event/Nonconformity" (EE/NC) reporting process. The appropriate corrective actions to address any such events will be assessed by the QA Manager in consultation with the Project Manager and with the Town(s). The QA Manager will generate and/or review all corrective actions required during the project and monitor their effectiveness in meeting project quality objectives. The Project Manager will review these issues on a regular basis, but the QA Manager will bring serious issues to the Project Manager's attention immediately. The Project Manager will report any corrective actions to the Town(s) in project QA/QC Corrective Action Log. A copy of the QA/QC Corrective Action Log will be provided to MassDEP with the Synthesis Report.

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

C1.2.1 *Field Corrective Action*

Corrective action in the field may be needed when the sample frequency is changed (i.e., more/fewer samples, sample locations other than those specified in the Embayment Specific Study Plan), or when sampling procedures and/or field analytical procedures require modification due to unexpected conditions. The survey crew may identify the need for corrective action. The Project Manager and QA Manager will approve the corrective measure. The Chief Scientist will ensure that the survey crew implements the corrective action.

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

Corrective actions will be implemented and documented as follows:

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

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

C1.2.2 *Laboratory Corrective Action*

Corrective action in the laboratory is specified in laboratory SOPs and may occur prior to, during, and after initial analyses. Conditions, such as broken sample containers, may be identified during sample log-in or analysis. Following consultation with laboratory analysts and supervisory personnel, it may be necessary for the QA Manager to approve the implementation of a corrective action. If the problem makes it impossible to achieve project objectives, the laboratory manager will be notified, who will in turn notify the Project Manager. The Project Manager will communicate with other members of the project team or the Town(s), as necessary.

These corrective actions will be performed prior to release of the data from the contracted laboratory. The corrective action will be documented in both the laboratory's corrective action files, and in the data report generated by the laboratory. If the corrective action does not rectify the situation, the laboratory will contact the Project Manager or a designee, who will determine the action to be taken and inform the appropriate personnel.

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

The need for corrective action may be identified during either data validation or data assessment. Potential types of corrective action may include re-sampling by the survey crew or reanalysis of samples by the laboratory. These actions are dependent upon the ability to mobilize the survey crew and whether the data to be collected are necessary to meet the required QA objectives. If the data validator or data assessor identifies a corrective action situation that impacts the achievement of the project objectives, the Project Manager will be responsible for informing the appropriate personnel, and the Town(s).

C2. REPORTS TO MANAGEMENT

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

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

A summary of QA issues, audit findings, and significant nonconformities will be included in the project QA/QC Corrective Action Log submitted to the Town(s).

D. DATA VALIDATION AND USABILITY

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

- Data verification/validation
- Data usability assessment

D1. DATA REVIEW, VERIFICATION, AND VALIDATION

D1.1 Field Data

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

D1.2 Laboratory Data

Prior to the release of any data from a contracted laboratory, the data will be reviewed and approved by laboratory personnel. The review will consist of a tiered approach (Section D2.2) that will include reviews by the person performing the work, by a qualified peer, and by supervisory and/or QA personnel.

D1.3 Data Management

The review process will include verification of manually entered data and QC checks run in a software application prior to submitting the data to MassDEP and the database. Detailed descriptions of these processes are included in Sections B10 and D2.

D2. VALIDATION AND VERIFICATION METHODS

D2.1 Field Data

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

- Logbooks and standardized forms have been filled out completely and that the information recorded accurately reflects the activities that were performed.
- Records are legible and in accordance with good recordkeeping practices, *i.e.*, entries are signed and dated, data are not obliterated, changes are initialed, dated, and explained.

- Equipment calibration, sample collection, handling, preservation, storage, and shipping procedures were conducted in accordance with the protocols described in the QAPP, and that any deviations were documented and approved by the appropriate personnel.
- Verification that in-situ duplicate measurements and post-sampling calibration checks meet accuracy and precision data quality goals listed in Table 5.

D2.2 Laboratory Data

As part of data validation, each laboratory used will ensure that:

- The QC checks specified in Sections A7 and B5 were conducted and met the acceptance criteria.
- All data that are hand-entered (*i.e.*, typed) will be 100% validated by qualified personnel prior to use in calculations or submission to the Project Manager.
- All manual calculations will be performed by a second staff member to verify that calculations are accurate and appropriate.
- Calculations performed by software will be independently verified at a frequency sufficient to ensure that the formulas are correct, appropriate, and consistent, and that calculations are accurately reported.

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

D2.3 Data Management

Laboratory data will be reviewed by the Project Manager prior to the electronic submission to MassDEP and the selected database. Data review may include methods such as plots, logical checks, and range checks to identify suspect values. Routine system back-ups are performed daily. Data provided electronically to facilitate data handling will be verified against the hard copy data. Detailed description of data management and review is provided in section B10 of this QAPP.

D2.4 Project Deliverables

Upon completion of the verification/validation process, a dataset packet will be prepared for submittal to the Town(s) and MassDEP. This documentation will include the following elements required for MEP benthic monitoring and as listed in Section A9.4.

- Cover letter that includes a description of any problems
- List of problems encountered, and corrective actions taken
- List of samples/images planned vs. collected, or measurements planned vs. reported
- Quality Assurance Statement including a checklist of QA actions, and notes on deviations and corrective actions
- Table(s) of data submitted

D3. RECONCILIATION WITH USER REQUIREMENTS

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

The data generated must meet the MEP's needs as defined in the project DQOs defined in Section A7 of this QAPP. The primary objectives for assessing the usability of the data are to ensure that (1) data denote conditions and habitat quality in the embayment being studied, (2) all datasets are complete and defensible, and (3) data are of the quality needed to meet the overall objectives of the MassDEP.

D3.1 Comparison to Measurement Criteria

D3.1.1 *Accuracy and Precision Assessment*

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

D3.1.2 *Completeness Assessment*

Completeness is the ratio of the number of valid sample results to the total number of results planned for collection. The goal of this program is to generate valid, usable data. However, in environmental sampling and analysis, some data may be lost due to sampling location logistics, or field or laboratory errors. The overall completeness goal for the MEP Marine Benthic Monitoring Program is 100% of planned samples to be collected and analyzed. The Project Manager will assess the completeness of the overall data generation against the project goals.

Following completion of the sampling, analysis, and data review, the percent completeness will be calculated and compared to the project objectives stated in Section A7.2 using the following equation.

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

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

D3.1.3 *Representativeness*

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

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

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

D3.2 Overall Assessment of Environmental Data

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

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APPENDIX A

MEP Marine Benthic Monitoring
Field Standard Operating Procedures

Disclaimer: All applicable federal, state, and local laws and regulations are to be followed when conducting activities described in this Standard Operating Procedures. The Massachusetts Department of Environmental Protection and the Commonwealth of Massachusetts accept no responsibility and no liability for loss of any kind, including personal injury or property damage due to the work and/or activities described in this Field Standard Operating Procedures.

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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 dissolved oxygen) to highly stressed (high organic matter, low dissolved oxygen). 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 2020). 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 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.

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

2.0 Technical Approach

MEP benthic monitoring will include sampling to support a sediment and benthic macroinvertebrate community characterization. The approaches to conducting sampling for this characterization are described below in Sections 2.1 (water quality), 2.2 (soft-bottom infaunal and sediment), 2.3 (under digital still images and/or video), 2.4 (hard-bottom/ riprap destructive), and 2.5 (soft-bottom sediment profile imagery). All MEP benthic monitoring will be conducted in August-October. Sampling in this period is consistent with previous MEP benthic monitoring procedures and the National Coastal Condition Assessment (NCCA). Benthic sample locations in previously assessed embayments will be determined by MassDEP using existing benthic sampling locations and the process

described in the MEP Benthic Monitoring QAPP. Benthic sample locations in unassessed embayments will be determined following a Generalized Random Tessellation Stratified (GRTS) sampling design as described in the MEP Benthic Monitoring QAPP. Sample locations for all monitoring approaches will be identified by latitude and longitude (decimal degrees) in the Embayment Specific Study Plan and approved by MassDEP prior to field activities.

All ecological sampling activities performed by the Town or town representatives (Town staff, seasonal employees, volunteers, and/or contractors) for MEP benthic monitoring will be conducted following the provisions of: (1) a Massachusetts Division of Marine Fisheries Scientific Collector's Permit, and (2) any local permits that are required.

The Chief Scientist must notify the Project Manager and MassDEP, Town or teaming partner before any unanticipated changes to the survey sampling occur. Examples of unanticipated changes include, but are not limited to, an unanticipated barrier, mooring field, or eelgrass is encountered, any changes to sample locations or transects, and/or length and proximity of video sites. This notification requirement will ensure that the survey will be conducted as stated in the Survey Plan and the selected procedures in this Field SOP unless there is a compelling reason for modification. The notification will also facilitate a brief discussion between the Chief Scientist, project manager, and MassDEP, the Town, or teaming partner, which will help ensure that possible consequences for data collection and data quality are considered.

2.1 Water Quality Measurements

Benthic macroinvertebrate community structure is influenced by water quality, including water temperature, dissolved oxygen (DO), pH and salinity. Water quality data will be used to understand the different communities that can be observed in an estuary and indicate areas that may have impairment. Water temperature, DO, pH, and salinity measurements will be taken at each station prior to soft-bottom infaunal, sediment, or hard-bottom destructive sampling with a calibrated multi-parameter water quality sonde. Measurements will be taken at specific depth intervals, consistent with the NCCA (See Section II), within 37 meters of the designated station location. Water quality data will be submitted via an electronic file.

2.2 Soft-Bottom Macroinfauna and Sediment Sampling

The benthic macroinvertebrate assemblage and the sediment (i.e., grain size and total organic carbon) present at stations selected throughout an embayment will serve as the basis for assessing embayment health. Sediment samples (for benthic macroinfauna and sediment characteristics) will be collected using a 0.04-m^2 Young-modified Van Veen grab. Benthic stations will be located and verified using marine navigation grade GPS with the coordinates provided in the Embayment Specific Study Plan. Along with the collection of the benthic samples, the field crew will also complete the field processing of those samples. Macrofauna samples will be gently washed through a 0.500 mm-mesh sieve and preserved

in 10% buffered formalin. Samples will be kept in the custody of the Chief Scientist and transferred with appropriate Chain-of-Custody forms to the laboratory for processing.

Additional grab samples will be collected for grain size analysis and total organic carbon at each of the sampling stations.

2.3 Digital Still Image and/or Video Monitoring

Underwater still images and video are used to document the current condition of an area and provide a landscape perspective on the benthic habitat with information including substrate characteristics, habitat relief, the occurrence of large identifiable taxa, species relationships, and human impacts (e.g., plastic trash, lost fishing gear, and fishing activities and effects). To achieve high quality underwater still or video images, the field crew will deploy the camera and record the still images or video prior to any bottom disturbing collection activity. A drop camera system will be deployed to record a minimum of 2 digital still image or 2 minutes of video of the bottom at each station prior to soft-bottom macroinfauna and sediment sample collection. For hard-bottom or riprap areas, a drop camera, towed camera, or SCUBA divers will be deployed to record digital still images or video of the bottom prior to all hard bottom/riprap destructive sample collection or as a stand-alone hard bottom survey. The Embayment Specific Study Plan will specify the underwater digital image approach or approaches to be used for embayment monitoring prior to field activities. Upon arrival to the station location, the depth will be verified using the onboard depth sounder and recorded on the appropriate datasheet.

Digital images (still or video) will be collected by a drop camera using a high definition (HD) digital camera. The drop camera will be attached to a frame that either captures a 1.0 m^2 quadrat in the view frame or has two attached scaling lasers with a range of 5 to 10 centimeters at 100 feet separation. Prior to deploying the drop camera, an image of a whiteboard with the date, time, and station number written on it will be taken. The camera will be lowered to an elevation 2 to 4 feet off the bottom. Turbidity can be a limiting factor; the final elevation will be determined on site at the time of the monitoring. The drop camera will be monitored in real time by a field biologist onboard the vessel to ensure quality is acceptable for a detailed review.

Digital still images can be collected by SCUBA divers using a HD digital camera with attached lights in conjunction with a 1.0 m^2 quadrat (e.g., aluminum, PVC). At each sampling location an image of a dive slate with the date, time, and station number written on it will be taken. The quadrat will be placed horizontally on the substrate and a minimum of two images will be collected at each sample location. Images will be taken perpendicular to the bottom, at a uniform distance from the surface across all samples. If turbidity causes poor visibility, a smaller quadrat (0.5 m^2) may be substituted with a minimum of 4 images collected at each location.

Digital video can be collected by SCUBA divers using a HD digital camera with two attached scaling lasers with a range of 5 to 10 centimeters at 100 feet separation. The

beginning of each video will include a shot of a whiteboard or dive slate with the date, time, and station number written on it. Digital video will be conducted along 100-foot transects. If a specific feature is being recorded, the transect will be centered on the feature and be parallel to its longest dimension. The camera will be maintained at a constant elevation off the bottom of 2 to 4 feet if possible. Turbidity can be a limiting factor; the final elevation will be determined on by the divers at the time of the monitoring.

All original field datasheets and associated digital image files will be generated by and remain in the custody of the Chief Scientist. Appropriate Chain-of-Custody forms will accompany the samples when transferred from the field to the laboratory for analysis.

Stand-alone Video Survey (Optional) – A stand-alone video survey will be conducted if proposed in the Embayment Specific Study Plan. Digital video will be collected by a towed camera unit, remotely operated underwater vehicle (ROV), or two SCUBA divers. The digital camera will have two attached scaling lasers with a range of 5 to 10 centimeters at 100 feet separation. The beginning of each video will include a shot of a whiteboard with the date, time, and station number written on it. At least 20 minutes of HD video footage will be obtained from each station or area. If transects are established and conducted using a camera system towed from a vessel, the vessel will move at approximately ¼ knot speed during video recording in order to maintain position along the transect. The slow vessel speed will enable clear, detailed, and reviewable video images to be obtained. If divers or a remotely operated underwater vehicle (ROV) are used to conduct the survey, the survey vessel will be anchored near the station to maintain position. Specific stations, areas, or transect locations and length will be determined in the Embayment Specific Study Plan prior to field activities. For transects, the transect length may extend beyond the area or feature of interest (e.g., hard-bottom or riprap boundaries); as doing so will enhance the overall understanding of the embayment. The tow camera unit will maintain a constant elevation off the bottom of 2 to 4 feet if possible. Turbidity can be a limiting factor; the final elevation will be determined on site at the time of the monitoring. The video will be monitored in real time by a field biologist onboard the vessel (except for surveys conducted by divers) to ensure quality is acceptable for a detailed review.

All original field datasheets and associated digital image files will be generated by and remain in the custody of the Chief Scientist. Appropriate Chain-of-Custody forms will accompany the samples when transferred from the field to the laboratory for analysis.

2.4 Hard-Bottom/ Riprap Destructive Sampling (Optional)

Hard-bottom or riprap destructive sampling will be conducted if proposed in the Embayment Specific Study Plan to quantitatively characterize the macrofauna and macroflora found on hard surfaces in the embayment. Hard-bottom or riprap destructive sample locations will be determined by GRTS sampling design as described in the MEP Benthic Monitoring QAPP and identified in the Embayment Specific Study Plan prior to field activities.

Destructive samples will be collected by divers using a 1/16 m^2 frame placed horizontally on the hard substrate and an airlift dredge. The diver will take a digital photograph with an underwater camera (e.g., GoPro, Sony), and note total percent cover of macroalgae found within the frame and any finfish observed in the area before sample collection. Total percent cover of macroalgae will be characterized using the modifiers defined in the Coastal and Marine Ecological Classification Standard (CMECS) presented in Table 1. The sample will then be collected into a 0.79 mm mesh bag. The square is to be vacuumed at a high suction rate to capture the highly motile organisms, then large organisms such as echinoderms and algae will be picked by hand and placed into the bag. The area within the frame will then be cleaned of all organisms. The bag will be removed, tied, returned to the surface, and the sample preserved in 10% buffered Formalin. Samples will be kept in the custody of the Chief Scientist and transferred with appropriate Chain-of-Custody forms to laboratory staff for processing.

Table 1. Percent Cover Modifiers (FGDC 2012).

Coarse Percent Cover Values	**Fine Percent Cover Values**
Trace	< 1%
Sparse (1 to < 30%)	1 to < 10%
	10 to < 20%
	20 to < 30 %
Moderate (30 to < 70%)	30 to < 40 %
	40 to < 50 %
	50 to < 60 %
	60 to < 70 %
Dense (70 to < 90%)	70 to < 80 %
	80 to < 90%
Complete	90 to 100%

2.5 Soft-Bottom Sediment Profile Image (Optional)

Soft-bottom Sediment Profile Imagery (SPI) will be conducted if proposed in the Embayment Specific Study Plan. SPI sample locations in previously assessed embayments will consist of all of the existing benthic locations sampled during the initial assessment found in the embayment technical report and re-identified in the Embayment Specific Study Plan. SPI sample locations for unassessed embayments will be determined by GRTS sampling design as described in the MEP Benthic Monitoring QAPP and identified in the Embayment Specific Study Plan.

A sediment profile camera system will collect at least three photographic images for analysis from each station generally within the first 12 seconds after bottom contact. The sediment profile images will be reviewed and the following parameters evaluated: sediment grain size; sediment layering, thickness, and type; surface and subsurface fauna and

structures; approximate prism penetration depth; approximate surface relief; approximate apparent redox potential discontinuity (aRPD; the transition from oxidized to reduced sediment conditions) depth, and other major, readily discernable patterns. The aRPD depth will be categorized using the CMECS depth modifiers (Table 2). All original SPI field datasheets and associated video and digital image files will be generated by and remain in the custody of the Chief Scientist. Appropriate Chain-of-Custody forms will accompany the samples when transferred from the field to the laboratory for analysis.

Table 2. 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

3.0 Sampling Schedule and Logistics

Benthic sampling will be conducted during August-October of a sampling year. Embayment specific surveys and sampling locations will be determined and described in the survey plan section of the Embayment Specific Study Plan following the MEP Benthic Monitoring QAPP prior to field activities. The embayment specific survey plan will detail the schedule of operations, field team, vessel, benthic monitoring surveys to be conducted, sequence of tasks and events, sampling location and coordinates of each station, and sample handling and custody.

4.0 Sample Site Selection

Benthic sample locations in previously assessed embayments will be determined by MassDEP using existing benthic sampling locations and the process described in the MEP Benthic Monitoring QAPP. In unassessed embayments, benthic sample locations will be determined following a GRTS sampling design as described in the MEP Benthic Monitoring QAPP. The number of sampling locations will depend on the approach and tier selected for each embayment. Sample locations will be identified in the survey plan section of the Embayment Specific Study Plan prior to field activities. Preceding each survey, the field team will receive a listing of sampling locations that includes the Site ID, Sample ID, Latitude and Longitude for each location, along with a map of sampling locations. The list of sampling locations will include Alternate locations that will be used if sampling cannot be conducted at the Primary locations.

A review of access to sample locations in previously assessed embayments or a site evaluation for unassessed embayments will be performed prior to the start of field work to identify issues with boat access to small or narrow sub-embayments. An access review will provide an opportunity to identify any problems with access before sampling and allows for a discussion with MassDEP, the Town, or teaming partner to finalize the stations in the area and possible alternative locations. The site evaluation will ensure sampling locations and alternative locations meet the target population identified in the selected GRTS survey design. The evaluation will also provide initial verification of site suitability and further align the MEP benthic monitoring protocols with the National Coastal Condition Assessment and Massachusetts Coastal Condition Assessment programs. A desk study is recommended for both the access review and the site evaluation to help minimize costs.

Sampling sites will be located by the field team using shipboard differential Global Positioning Systems (GPS).

In general, water quality, benthic, and sediment samples will be collected within a 37 m radius of the predetermined sample locations (point X). However, if grab samples cannot be collected at point X, they can be collected at another location within the 37 m radius of point X.

If acceptable grab samples cannot be collected within the 37 m radius, they may be taken in the >37 m to 100 m radius area of point X without the need to resample the water quality. If sediment samples still cannot be obtained after 3 attempts within the 100 m radius of point X, that site location will be designated as "completed, no sample", and noted as such on the field data sheet.

5.0 Quality Assurance

The MEP has developed a Quality Assurance Project Plan (QAPP) that presents the organization, objectives, functional activities, and specific quality assurance (QA) and quality control (QC) activities associated with the Benthic Monitoring. The QAPP describes the specific protocols that will be followed for sampling, sample handling and storage, chain of custody, laboratory and field analyses, data review and validation, document management, data management, and data usability assessment. Aspects of QC and QA procedures from the QAPP are summarized below.

5.1 Quality Control

The function of QC is to continually monitor the reliability and validity (accuracy, precision, and completeness) of data produced on a daily basis. The QAPP has been approved by MassDEP and any changes to the procedures must be coordinated with MassDEP through the current Massachusetts Estuaries Project Manager. For the Benthic Monitoring, Chief Scientists will act as quality control supervisors who will:

- Monitor performance and results of quality control procedures;
- Monitor instrument maintenance, calibration, and reliability;

- Monitor sample control procedures and documentation; and
- Monitor training of technicians.

Specific sample control procedures including packaging, preservation and Chain-of-Custody (COC) for each task are documented in Sections II through VI of this SOP. In general, each sample is given a unique sample number. Each sample is then tracked by its sample number from field collection and throughout the laboratory and data processing functions. Daily collection of samples is tracked from the field site to the laboratory for final analysis by means of a COC form. At the laboratory each sample is tracked through each storage and analysis step by means of sample control logs. The function of this process is to provide a paper trail of who performed each step in the analysis of a sample from collection to storage, when each step occurred, what condition the samples were in and where each step took place.

Data Control Procedures

Specific data control procedures for completing datasheets and COC forms are documented in Section VII Data Handling of this SOP. In general, all completed datasheets are maintained by the Project Manager. The project field files contain the following documentation:

- Embayment Specific Study Plan,
- Field Standard Operating Procedures,
- Copies of all datasheets,
- Sample and data logs.

Training of Technicians

To assure the standardization of field procedures, a two-level system for training field team members will be used. The first level is documented standard operating procedures, and the second level is a training program for all new project personnel. At a minimum, this training program consists of:

- A complete reading and explanation of the project QAPP and field SOP.
- The Field Manager will observe the first two or more times a new procedure is performed.
- Personnel assigned to unfamiliar tasks are accompanied by an experienced team member for at least their first two attempts.

5.2 Quality Assurance

The function of QA is to verify the achievement of quality through all phases of a project. This is accomplished primarily by audits, tests, and surveys that provide objective evidence that quality control programs are being implemented. Field tasks are subject to at least one audit per sampling year. The audit is conducted by a QA Manager, who reviews the operations, plans, and survey objectives and examines the acquisition and transfer of data from the field to the laboratory.

Audit results are presented to the appropriate project management by the QA Manager after the audit has been completed. At this time, specific findings are presented, and recommended courses of corrective action developed. The audit results are documented in a written report and reviewed by the Project Manager and Chief Scientist that have responsibility in the area audited. These reports include a summary of audit results, observations made with a listing of non-conformities, recommendations and corrective action taken.

The QA Manager and Project Manager maintain files of all project audits. This file includes copies of the audit checklists, audit reports, written replies, the record of completion of corrective action and follow-up action.

5.3 Non-Conforming Items and Corrective Action

Documentation of problems or unusual events occurring during a survey is accomplished using Extraordinary Event/Nonconformity (EE/NC) Report. The EE/NC Report is designed to dispense information to the Project Manager and the QA Manager and to obtain necessary action on items that are critical to technical operations. The EE/NC Report is used to describe results from observations such as:

- Losing a sample
- Noting samples that are grossly different from expected (content, preservation, labels)

The EE/NC Report is designed for use by any person who identifies a problem. The originator's supervisor is responsible for delivery of the completed form to the Project Manager. The Project Manager must respond within ten working days. The contracted QA Manager is informed of each report and maintains an awareness of the status of follow-up.

Samples or data not in conformity with specifications or which do not meet preconditions for the next step in processing or use, are set aside until the problem is resolved and documented via the EE/NC procedure detailed in the MEP Benthic Monitoring QAPP.

EXTRAORDINARY EVENT/NONCONFORMITY REPORT

EE/NC Report Number:

Project Name: _____

Date: _____

Originator: Name: _____

Problem: _____

Recommendation: _____

_____

Signature: _____ Date: _____

Project Manager: Name: _____

Recommendation: _____

_____

_____

Signature: _____ Date: _____

Quality Assurance: Name: _____

Response: _____

_____

Signature: _____ Date: _____

Project Manager: Name: _____

Report Filed: _____

Signature: _____ Date: _____

Distribution List of Copies: _____

_____

Figure 1. Extraordinary Event/Nonconformity Report.

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.

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.

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.

US EPA (U.S. Environmental Protection Agency). 2020. National Coastal Condition Assessment 2020 Field Operations Manual. April 2020. EPA- 841-F-19-005. U.S. Environmental Protection Agency, Office of Water, Office of Wetlands, Oceans and Watersheds. Washington, DC. 149 pp.

II. Field Standard Operating Procedures for Water Quality Measurements

Measurements for water temperature, dissolved oxygen (DO), pH, and salinity will be taken at each station prior to soft-bottom infaunal, sediment, or hard-bottom destructive sampling using a calibrated multi-parameter water quality sonde. At the start and end of each sampling day the multi-parameter sonde will be calibrated following the manufacturers' recommended procedures for each probe. Sonde measurements at the end of each sampling day are checked against the calibration standards and used to assess the accuracy of the instruments performance as described in NCCA Field Operations Manual Section 6.3 (US EPA 2020). The calibrations will be recorded on a Calibration Log (Figure 2). If the water quality sonde manufacturer provides a calibration worksheet, use the manufacturer's worksheet to document the calibration. Water quality will be submitted by the Chief Scientist by an electronic file.

1.0 Equipment List

Water quality measurements will be taken with a calibrated multi-parameter sonde. The following is the supply list for collecting measurements:

- Multi-parameter water quality meter with a data recorder and temperature, DO, pH, and salinity/conductivity probes
- Extra batteries

Following is the supply list for calibrating the multi-parameter sonde:

- De-ionized water
- Calibration cups
- Calibration standards, solutions, and/or buffers
- Thermometer
- Barometer to use for calibration

2.0 Gear Deployment

The following describes the sampling procedure to obtain water temperature, DO, pH, and salinity consistent the NCCA Field Operations Manual (US EPA 2020).

1. Use the vessel instrumentation to measure the total water depth at the station to the nearest 0.1 m and record on the Sample Collection form (See Section III, Figure 4). The hydrographic profile data will be electronically recorded and submitted by an electronic file.
2. Lower the sonde into the water and record single water temperature, DO, pH, and salinity measurements at each depth after all readings have stabilized. Measurements will be collected at the following depths: 0.1 m below the surface, 0.5 m below the surface, every 1 m from depths of 1.0 to 10.0 m, and if the site is deeper than 10 m, every 5 m thereafter. Take the last set of measurements at 0.5

m from the bottom, making sure to not let the sonde touch the bottom. If the sonde touches the bottom wait for the sediment disturbance to settle for a minimum of 5 minutes.

3. The field crew will note any questionable measurements and retake the water quality measurements at the same depth interval as the probe is retrieved (upcast). The second measurements will be used to verify the initial readings.
4. The field crew will note any measurements that need further comment or when a measurement cannot be made. The explanation of these notes will be placed on the Sample Collection form.

3.0 Form

Calibration Log

Date	Time	Equipment	Initials
	Present	**Should Be**	**Adjusted To**
Battery level			
Temperature °C	(Instrument =I)	(Certified therm. = T)	**ΔT=I - T**
Barometric Pressure			
Dissolved Oxygen	present reading mg/L %	 100%	 mg/L %
Conductivity		50.00	
pH		7.00	
		10.00	

Figure 2. Example of a Calibration Log.

III. Field Standard Operating Procedures for Soft-Bottom Infaunal and Sediment Sampling

Soft-bottom benthic sample collection will be conducted in the following order:

1) Collect water quality measurements (See Section II);
2) Deploy drop camera and take digital images of the bottom (See Section IV);
3) Collect macrofauna sample;
4) Collect sediment sample for grain size and total organic carbon (TOC)

1.0 Equipment List

A 0.04-m^2 Ted Young-modified Van Veen grab sampler will be used to collect soft-bottom sediment samples for benthic macrofauna and sediment characteristics (grain size and TOC) analyses. Following is the supply list for collecting samples:

- Young-modified Van Veen grab with grab stand or frame if needed
- Weights and pads for grab
- Nitrile gloves
- Plastic tub or bucket
- 0.500 mm screening bucket or stainless steel sieve
- Sieve box or bucket
- Electrical tape
- Forceps (fine-tipped)
- Funnel (wide-mouth)
- Formalin[6,7] (37% formaldehyde)
- Borax[8]
- Ruler (cm)
- Squirt bottle (ambient water)
- Stainless steel mixing pot or bowl with lid
- Stainless steel or Teflon spoons (15”), scoops, or spatula
- Glass, Nalgene or other sturdy plastic, wide-mouth sample jars (500 ml) with lids
- Glass, Nalgene or other sturdy plastic, wide-mouth sample jars (125 ml) with lids
- Plastic bags (e.g., Whirl Pak, for grain size samples)
- Scrub brush

[6] Follow safe chemical handling procedures including: the use of personal protective equipment (e.g. gloves and safety glasses), the manufacture’s Safety Data Sheet recommendations, and the OSHA Formaldehyde Standard (29 CFR 1910, 1048) when using this chemical.
[7] A prepared 10% buffered formalin solution can be used for samples and purchased through laboratory chemical suppliers.
[8]Borax is necessary as buffer only if you are preparing your own 10% buffered formalin solution.

- Cooler with wet ice.

The following items will also be needed for recording measurements:

- Survey Log form (Figure 4)
- Sample Collection form (Figure 5)
- #2 Pencils
- Waterproof paper for internal sample jar labels
- Fine-tipped indelible markers
- Write-on yellow tape or pre-printed write on labels
- Clear tape strips

2.0 Gear Deployment

The Van Veen grab sampler will be used to collect sediment samples for benthic macrofauna and sediment characteristics. Three grab samples (2 to be sorted and 1 archived) for macrofauna and one grab sample for sediment characteristics will be collected from each benthic station.

Once the survey vessel is on station and coordinates have been verified, the water quality and underwater video surveys will be performed (see Sections II and IV). Once these surveys have been completed, the sediment grab will be deployed. When slack in the winch wire indicates that the grab is on the bottom, the grab and captured sample will be brought back to the surface. Upon retrieval of the grab, the sample will be inspected for acceptability (sufficient quantity, undisturbed surface layer, not washed out; Figure 3). If the sample is unacceptable, the grab will be emptied, rinsed, and redeployed.
Precautions will be taken during the deployment and retrieval of the grab sampler to prevent contamination of samples between stations. Sampling for infauna, TOC, and grain size all require that the grab and associated sampling equipment be washed and thoroughly rinsed between sampling stations.

The following describes the sampling procedure to obtain sediment samples as outlined in National Coastal Condition Assessment (NCCA) Field Operations Manual (US EPA 2020).

Note: *The sampler, spoons and mixing bowl or bucket must be thoroughly rinsed with ambient water after sampling at each site to ensure no sediments remain. This practice reduces the risk of the equipment carrying residues from site to site.*

1. Attach the sampler to the end of the winch cable with a shackle and tighten the pin (or secure the pin with a cable tie).
2. Set the grab according to the manufacturer's instructions and disengage any safety device designed to lock the sampler open.

3. Lower the grab sampler through the water column such that travel through the last 5 meters is no faster than about 1 m/sec. This minimizes the effects of bow wave disturbance to surficial sediments.
4. Allow a moment for the sampler to settle into the substrate and then allow slack on the cable (letting the cable go slack serves to release the jaws of the sampler so they will close as the sampler is retrieved).
5. Retrieve the sampler and lower it into its cradle or a plastic tub on-board. Open the top and determine whether the sampling is successful or not (see Figure 3).
    - A successful grab is one having relatively level, intact sediment over the entire area of the grab, and a sediment depth at the center of at least 7 centimeters.
    - Grabs containing no sediment, partially filled grabs, or grabs with shelly/rocky substrates or grossly slumped surfaces are unacceptable.
    - Grabs completely filled to the top, where the sediment is in direct contact with the hinged top, are also unacceptable.
    - It may take several attempts using different amounts of weight to obtain the first acceptable sample. More weight will result in a deeper bite of the grab. In very soft mud, pads may be needed to prevent the sampler from sinking into the mud. If pads are used, the rate of descent near the bottom should be slowed even further to reduce the bow wave.
6. If, after several attempts, only grabs less than 7 centimeters deep can be obtained, use the next successful grab regardless of the depth of sediment at the center of the grab.
    - Use the comments section on the Sample Collection form (Figure 5) to describe your efforts and be sure to accurately record the depth of the sediment captured by the grab.
7. Carefully drain overlying water from the grab. If the grab is used for benthic community analysis, the water must be drained into the container that will receive the sediment to ensure no organisms are lost.
8. Enter notes on the condition of the sample (smell, substrate, presence of organisms on the surface, etc.) in the Sediment Characteristics section of the Sample Collection form.
9. Process the grab sample for either benthic community analysis or sediment testing as described below.
10. Repeat steps 3-9 until all samples are successfully collected. To minimize the chance of sampling the exact same location twice, the boat engines can be turned periodically to change the drift of the boat, or additional anchor line can be let out.

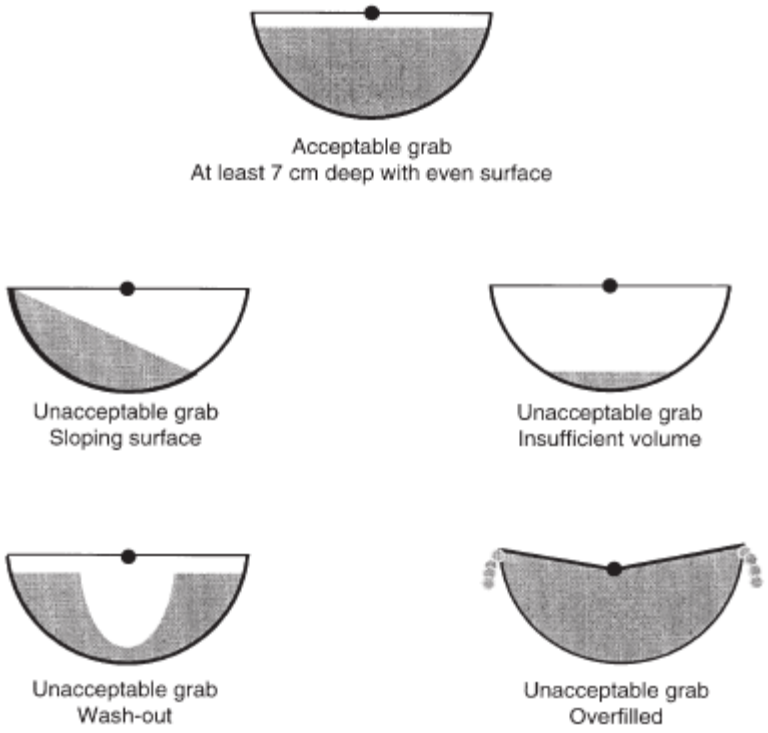

Figure 3. Illustration of acceptable and unacceptable grabs for benthic community analysis using a 0.04m^2 Van Veen grab sampler (from US EPA 2015).

3.0 Collection Procedure

3.1 Field Processing of Benthic Macrofauna Samples

Prior to sample collection (before the sample jar gets wet), an external label (including station location, replicate number, and date) will be taped to the outside of the jar. The external label may be pre-printed and taped on using clear tape or written directly on write-on yellow tape or a pre-printed adhesive label. Sample jars will be pre-filled with concentrated preservative solution by placing 1 teaspoon of Borax and 125 mL of formalin into a 500 mL sample jar[9], following safe chemical handling procedures. The concentrated preservative solution will produce a 10% buffered formalin-seawater solution when the sample jar is filled with seawater.

If the macrofauna grab sample is acceptable, the sample will be processed. A field description of sediment is recorded following measurement of penetration depth. The sediment should be characterized as being coarse sand, fine sand, silt, clay, gravel, mud, or of a mixed type. The presence of shell hash should also be recorded. Obvious odors, such as hydrogen sulfide (the odor of rotten eggs), petroleum, other odors, or a lack of noticeable

[9] If a prepared 10% buffered formalin solution is used, sample jars to do not need to be pre-filled.

odors should be recorded. General sediment colors (i.e., black, green, brown, red, or gray) should also be recorded. Photographs of the grab may be taken before and after the sample has been sieved; Sample labels (station location, replicate number, and date) will be included in each photograph to identify the sample. The penetration depth of the sample will then be measured using a plastic ruler (marked in millimeters) pushed into the sediment. Any sediment adhering to the surface of the ruler will be rinsed back into the grab for processing with the remainder of the sample. The grab will then be placed over a 0.500 mm screening bucket or sieve, the jaws opened, and the sample emptied into the bucket or sieve. Clean seawater will be used to gently rinse the sample. Large macrofauna or epifauna will be removed and not counted in the sample total (e.g., sand dollars, anemones, shrimp, sea cucumbers, snails, clams, mussels, and hermit crabs). The portion retained on the screen will then be transferred into labeled jars and fixed in 10% buffered formalin in seawater. The sieve will be carefully inspected to ensure that all organisms were removed. A rinse bottle will be used to rinse the outside of the sample bottle onto the screen to capture any organisms that did not make it into the mouth and stuck to the side of the sample jar. Fine forceps can be used if necessary to transfer infauna to the sample jar. Sample jars will be glass, Nalgene or other sturdy plastic jars with screw-capped lids. Using a #2 pencil, fill out an internal label on waterproof paper including station location, replicate number, and date and place in the jar with the sample. Each sample jar will be filled no more than half full of sample material. The sample bottles should then be filled with seawater to the top to achieve the correct preservation dilution and to prevent sloshing during transport, which can result in organisms getting stuck to the cap. If a pre-prepared 10% buffered formalin solution is used, the sample jar should be filled to the top with the prepared solution to prevent sloshing. The jar will be gently turned around on its side to distribute the buffered formalin evenly throughout the sample. The screening bucket or sieve will be washed between samples using copious amounts of forceful water and a stiff brush.

All benthic macrofaunal samples must be handled gently during the sieving process, fixed in 10% buffered formalin as quickly as possible to prevent deterioration of the fauna, and all sample jars must be labeled accurately.

Following each benthic survey, the macrofaunal samples (stored in sturdy coolers) will be driven to the contracted laboratory. The samples to be processed, while still preserved in 10% buffered formalin, can be shipped by FedEx ground or 2-day express delivery (if courier delivery is not feasible). The lids on the plastic sample jars will be taped and the jars inserted individually into large zip-locked or tied plastic bags lined with absorbent padding.

3.2 Field Processing of Sediment Samples

If the grab sample to be used for sediment characteristics (grain size analysis and TOC) meets the acceptability criteria, the water overlying the sample will be siphoned or gently decanted from the grab. The surface sediment (0 to 2 cm) will then be collected with a scoop and transferred to appropriate storage containers provided by the laboratory. Laboratory instructions regarding fill levels and sample handling will be followed. If it is necessary to

collect more than one grab to obtain sufficient material for analysis, the grabs will be homogenized before subsampling. The sediment samples must be kept cold or frozen. All samples will be kept on ice in coolers during transport. Sample labels will be checked to ensure that all station information is complete and matches the field datasheet and COC.

Grain Size Analysis
Use a scoop to collect the upper 0–2 cm from the grab, homogenize, and collect approximately 50 ml subsample for grain size analysis. Place sediment into a clean, labeled, 125 ml wide-mouth jar (4 oz) or plastic bag. Samples once transferred to the laboratory can be refrigerated for up to 28 days.

TOC
Use a scoop to collect the upper 0–2 cm from the grab, homogenize, and collect approximately 50 ml for TOC. Place sediment into a clean, labeled, 125 ml wide-mouth jar (4 oz). Samples once transferred to the laboratory can be frozen for up to 28 days.

4.0 Sample Form

The Chief Scientist may retake a sample if he/she/they feels any problems have prevented a valid sample.

For grab samples, a waypoint will be entered into the shipboard GPS when a sample is collected. The marker set for each waypoint will be named as the station name, with the replicate number appended. Waypoints will be stored separately for the grab survey. A QC check of waypoints against the recorded coordinates will be done after each sample is collected. Waypoints will be stored on the shipboard GPS until data checking confirms that all samples were collected within 37 meters of the target station location. Any sample coordinates found through data checking to be outside of the 37-meter station radius will be compared against the sample coordinates for the stored waypoint. Thus, if an incorrect waypoint is identified through data checking, the hand-entered data will be compared to the electronic waypoint on the GPS, and any error discovered in the navigational data will be corrected as necessary.

At the beginning of each station, the date, arrival time, weather, and names of all field crew members present will be entered (see Survey Data Sheet; Figure 4). Measurements made and samples collected will be recorded on pre-printed Sample Collection Forms (Figure 5).

Information specific to sample collection will include:

- Station name
- Sample identification number
- Time and date of sample collection
- Sample description (color, texture, etc.)
- Samplers' initials
- Requested analyses

- Location (the latitude and longitude coordinates where a sample is collected).

MASSACHUSETTS ESTUARIES PROJECT: BENTHIC SURVEY DATA SHEET

Embayment Name:		**Time (Arrive/Depart)** /
Date:	**Weather:**	**Sea State:**
Station ID:	**Crew:**	**Chief Scientist:** **QA/QC confirm** ☐

Water Quality: Water Temp, DO, pH, Salinity

Time: _____ **Calibration Completed** ☐ **Surface to** _____ **(bottom depth m)**

Profile Taken ☐ **Number of Measurements** _____

Survey Type:

Digital Video ☐

Digital Still ☐

Soft-bottom Benthic Grab ☐ **Number of Samples Taken** _____

Samples taken within 37 m of station location? Y/N (If no, provide information below)

If GRTS survey, include information on "oversample" locations below.

Other ☐

Notes

Figure 4. Example of a Benthic Survey Data Sheet.

Sample Collection Form: Soft-Bottom Infaunal and Sediment Grab Samples

MEP Project Name:

STATION: ________________ TIME ON STATION:__________________ STATION DEPTH (M):__________________DATE:____________________	Weather:__________________________ Recorded By:__________________________
Sample data	**Field Measurements**
Sample ID:	Grab Size: 0.04-m^2
Latitude:	Grab Penetration (cm):
Longitude:	Sediment Texture:
Replicate:	Other:
Time:	Analyses: (circle all applicable) GR TOC FA
Sieved By:	Organisms observed:
Comments:	
Sample ID:	Grab Size: 0.04-m^2
Latitude:	Grab Penetration (cm):
Longitude:	Sediment Texture:
Replicate:	Other:
Time:	Analyses: (circle all applicable) GR TOC FA
Sieved By:	Organisms observed :
Comments:	
Sample ID:	Grab Size: 0.04-m^2
Latitude:	Grab Penetration (cm):
Longitude:	Sediment Texture:
Replicate:	Other:
Time:	Analyses: (circle all applicable) GR TOC FA
Sieved By:	Organisms observed :
Comments:	
Sample ID:	Grab Size: 0.04-m^2
Latitude:	Grab Penetration (cm):
Longitude:	Sediment Texture:
Replicate:	Other:
Time:	Analyses: (circle all applicable) GR TOC FA
Sieved By:	Organisms observed :
Comments:	
GR = grain size, TOC= total organic carbon, FA = Infauna	

Figure 5. Example of a Sample Collection Form.

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

Digital images will be taken before benthic grab sampling is conducted. Digital still or video images for a single sampling location will be accomplished by using a drop camera or two SCUBA divers. SCUBA divers (optional) are recommended in more turbid waters and areas with riprap due to their maneuverability compared to a towed system. A high definition (HD) digital camera in an underwater housing with either scaling lasers or lights with a 5 to 10 cm (2 to 4 inches) separation or a visible quadrat will be used. Sampling locations will be determined in the Embayment Specific Study Plan prior to field activities. If SCUBA divers are used to obtain digital still images the HD digital camera will be used in conjunction with a 1.0 m^2 quadrat placed horizontally on the substrate. SCUBA divers will follow safe diving practices during field activities.

A stand-alone digital video survey (optional) will be accomplished by the use of a towed underwater camera, a ROV, and/or two SCUBA divers using a HD digital camera in an underwater housing with scaling lasers or lights with a 5 to 10 cm (2 to 4 inches) separation. Digital video will be conducted along 100-foot transect centered on the hard-bottom feature and parallel to its longest dimension. Transect length will be determined in the Embayment Specific Study Plan prior to field activities. At each station, simultaneous high-definition video (1080 p) and still images will be captured with the digital camera (e.g., GoPro Hero 4).

To achieve high quality underwater still images or video the field crew will deploy the camera and record the video or still images before collecting soft-bottom infaunal, sediment, or hard-bottom/riprap destructive samples. Avoid heavy disturbance of the bottom with anchors prior to capturing the video and still images. Camera lenses will be checked for debris and water droplets and carefully cleaned using a soft absorbent cloth after each time the waterproof case is opened to replace batteries or download images. In addition, in-field video or still image review will occur in a shaded or darkened area to help improve screen visibility and reduce glare from sunlight. A computer monitor or large screen is recommended.

1.0 Equipment List

- GPS unit, either vessel navigation system or handheld
- Survey boat
- Camera frame or sled (for a drop or towed camera system)
- SCUBA equipment (for diver survey; includes 2 of each - compasses, depth gauges or dive computers, scuba tanks, buoyancy compensators with correct weights, regulators, masks and snorkels, fins, dive knives, and wetsuits)
- 2 small all-purpose buoys (for diver survey)
- Weighted lines (for diver survey)
- White board

- Dry erase markers
- Laptop computer
- Computer monitor (for a drop or towed camera towed system)
- Camera cable (100 feet; for a drop or towed camera system)
- 2 external hard drives (500 GB or larger in size)
- Video: High definition (HD) digital camera with underwater housing and strobes in video mode (use a low light camera if possible)
- Video: Two scaling lasers or lights
- Still Images: HD digital camera with an underwater housing (use a low light camera if possible)
- Still Images: 1.0 m^2 quadrat

2.0 Gear Deployment:

2.1 Drop or Towed Camera Systems

Video recordings are made using a HD digital camera in an underwater housing and strobes in video mode attached to a frame or sled with on-board computer monitor.

1) Obtain transect or station locations from the Embayment Specific Study Plan prior to video or still image survey.
2) Program geographic position system (GPS) points for each transect or station into GPS unit prior to the field effort and correct by Wide Area Augmentation System (WAAS) to an accuracy of ±3.0 – 3.5 meters.
3) Locate and verify each station location or the start and end points for each transect in the field with the GPS.
4) Attach the camera, lights, and scaling lasers to frame or sled and check the connection to the on-board computer monitor. Check all equipment for proper functioning. Check the camera battery life to ensure enough power is available to record the entire transect or station.
5) While the camera is still out of the water, start recording.
6) Image the white board with the date, time, and station at the start of video or still image recording at each transect or station.
7) Lift the frame or sled, gently place it over the side of the vessel, and slowly lower the system to the bottom. Confirm good image quality and distance from the bottom using the on-board computer monitor.
8) The camera system is held approximately one meter off the bottom to attain a field of view of one square meter. Turbidity can be a limiting factor; in areas of poor visibility the frame or sled can be lowered to maintain image clarity.
9) For video recording, the camera system will be towed at a speed of approximately 0.25 knot or the lowest speed possible to maintain course and direction and clear imagery.

10) For still images, the drop camera will be lowered and a minimum of 2 images of the bottom will be collected at each station. Images will be taken perpendicular to the bottom.
11) Upon completion of video or still image collection, slowly bring the camera system to the surface and lift the frame or sled back on board the vessel.
12) Review the video or images on the camera to ensure the data was saved correctly on the data card. Check the camera battery life and data card storage capacity.
13) Exchange batteries or data card as required between stations. Download data card immediately upon its removal from the camera to two external hard drives. One external hard drive will serve as the primary drive and be used to review the video or still images. The other drive will serve as a backup drive in case the primary drive becomes corrupted. Confirm files were properly saved to the external hard drives.

2.2 Divers Camera Use

1) Obtain transect or station locations from the Embayment Specific Study Plan prior to video or still image survey.
2) Program geographic position system (GPS) points for each transect into GPS unit prior to the field effort and correct by Wide Area Augmentation System (WAAS) to an accuracy of ±3.0 – 3.5 meters.
3) Locate and verify each station location or the start and end points for each transect in the field with the GPS.
4) Deploy weighted lines with buoys attached at each end from the survey boat between the points for each transect. The lines are weighted at each end and at varying intervals to minimize movement of the lines from the designated alignment between the beginning and end points and to prevent drift due to tides or currents. The lines are marked in 10-meter intervals.
5) Attach the lights to the camera and check all equipment for proper functioning. Check the camera battery life to ensure enough power is available to record the entire transect or station.
6) While the camera is still out of the water, image the white board with the date, time, and station or transect.
7) For video recording, two divers will swim each transect line maintaining a constant elevation off the bottom of 2 feet -4 feet at each transect. Turbidity can be a limiting factor; in areas of poor visibility the divers can lower their elevation to maintain image clarity. The camera is held approximately one meter off bottom to attain a field of view of one square meter.
8) Still images are collected using 1.0 m^2 quadrat placed horizontally on the substrate at pre-determined locations along each transect. A minimum of 2 images will be collected at each sample location. Images will be taken perpendicular to the bottom, at a uniform distance from the surface across all samples. Biological data and observations on

sediment type and other flora and fauna are recorded on a dive slate or with a similar method and then entered onto field datasheets.

9) After the divers have returned to the vessel, review the video or images on the camera to ensure the data was saved correctly on the data card. Check the camera battery life and data card storage capacity.
10) Retrieve the buoys and weighted lines once sampling is completed at the transect.
11) Exchange batteries or data card as required between stations. Download data card immediately upon its removal from the camera to two external hard drives. One external hard drive will serve as the primary drive and be used to review the video images. The other drive will serve as a backup drive in case the primary drive becomes corrupted.

Note: Special certifications are required for the SCUBA work contained in this SOP. All SCUBA diving conducted under this SOP is expected to occur under a Scientific Diving Program. SCUBA divers conducting digital image surveys are required to have a valid and current SCUBA certification from a recognized SCUBA certification organization (e.g., PADI, NAUI, or SSI) along with first aid, CPR/AED, and Emergency Oxygen for Scuba Diving Injuries (Divers Alert Network) certifications.

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3.0 Collection Procedure

The date and time will be recorded on the video image. Vessel start and finish positions at each survey location will be captured electronically using a navigational software program or handheld GPS unit. Transect and/or station ID, along with the date, vessel location coordinates, time, and water depth will also be recorded on the Station Log (Figure 6) at the start of each video recording or still image survey. At the end of each video recording or still image survey, the vessel location coordinates, along with the date, time, and water depth will be recorded on the Station Log.

4.0 Sample Form

At the beginning of each survey, the date, start time, weather, and names of all field team members present will be entered (see Survey Log Form, Figure 4). Measurements made and samples collected will be recorded electronically. Information specific to sample collection will include:

- Station name
- Sample identification number
- Time and date of sample collection
- Location (the latitude and longitude coordinates where a survey is conducted)

- Description of benthic habitat observed

Underwater Digital Image Survey **Station Log**		
MEP Project Name:		
Station:	**Date:**	**Survey (circle): Video / Still / Both**
Start Time:	**Start Depth:**	**Start Lat:**
		Start Long:
End Time:	**End Depth:**	**End Lat:**
		End Long:
Bottom Description:		
Notes:		
Video Minutes:		

Figure 6. Example of a Station Log for underwater still image and/or video monitoring.

V. Field Standard Operating Procedures for Hard-bottom/ Riprap Destructive (Infauna and Inflora) Sampling (Optional)

1.0 Equipment List

- 2 yellow mesh bags
- Field datasheets with coordinates
- Pencils
- 2 airlifts with 45º attachments
- (3) < 1.0 mm mesh bags per station and extras
- (2) 1/16 m^2 frames
- 2 small + 2 large sharp scrapers
- Clipboards
- Plastic bags
- Labels
- Survey Forms
- Cooler
- SCUBA equipment (includes 2 of each - compasses, depth gauges or dive computers, scuba tanks, buoyancy compensators with correct weights, regulators, masks and snorkels, fins, dive knives, wetsuits, and Shoe Goo)

Prior to use, all < 1.0 mm mesh bags should be checked for rips, all scrapers should be sharpened, and airlifts and hoses inspected for fitness. Bags with holes in the mesh should be mended or not used. Water quality measurements will be conducted at hard-bottom/ riprap destructive sampling locations before divers enter the water (See Section II above).

2.0 Gear Deployment

At benthic destructive stations, divers will proceed down the line to the bottom taking with them the following:

1. Airlift with 45-degree attachment and SCUBA tank.
2. 0.79 mm mesh bags with elastic cord for attachment to the airlift and appropriate labels and plastic bags for the mesh bag samples to be placed in.
3. Two sharp scrapers, 1 large, 1 small.
4. One clipboard with a Field Datasheet (Figure 7) which has directions (compass azimuth) and distances (number of swim kicks) from the starting location to each replicate. Directions use azimuths rounded to the nearest 5 degrees and number of kicks (1-12) are chosen using a random number table but are selected such that all but two of the five samples will be in different quadrants.
5. 1/16 m^2 frame.
6. Yellow mesh bags.

3.0 Collection Procedure

At the station the following procedures are followed:

1. The first replicate is located by going from the starting location along a specified compass azimuth for the specified kick strokes. A kick stroke is defined as a kick of the right foot in a normal kicking cycle and is closely equivalent to a distance of 1 meter. Care should be taken to avoid kelp and mussel beds.

2. The 1/16 m^2 frame is placed at the first replicate location utilizing the following guidelines:
   - The samples are to be as horizontal as possible.
   - Substrate heterogeneity is to be minimized as much as possible by selecting a flat, crevice free area covered with foliose algae within a 1/2 meter of the original random point.
   - If the original random point falls within an area scraped clear or a dense kelp bed or mussel bed, move back or forward along the specified compass azimuth to the edge of the bed to take the sample. Note the changed location on the field datasheet. Special care should be taken to avoid kelps.

3. Sampling is conducted in the following sequences:
   - The diver will take a digital photograph with an underwater camera and notes on total percent cover of macroalgae (except for the crustose algae) found within the frame, note any finfish observed in the area of sampling, and make appropriate comments on the condition of the sample. Percent coverage will be described using the modifiers provided in Table 2 of this document. The datasheet will be filled out. This is to be done without disturbing the sample area.
   - The sample is then collected into a < 1.0 mm mesh bag using the airlift dredge and a scraper. The square is first vacuumed at a high suction rate to capture highly motile organisms. Large organisms such as mussels, echinoderms and algae should be picked out by hand and placed in the bag. Large rocks should be discarded after all organisms are scraped from them. The area within the frame, including all crevices and rock sides to a depth of 4 in. should be cleaned of all organisms. The crustose algae should be chipped to expose pockets where animals, especially polychaetes, may reside. When sampling is completed, the bag is removed, tied, and placed in a plastic bag. The diver will squeeze as much water as possible from the plastic bag before tying it off. The bag will then be placed in a yellow mesh bag and secured to the airlift or the mooring block.
   - The scraped surface type is recorded on the Field Datasheet after the sample is collected.

- The diver must match the mesh bag with the proper internal tag that identifies the azimuth of the sample.

4. The remaining replicates are sampled following Steps 1, 2 and 3.

5. A general algae collection for voucher specimens of all macroalgae species at the station, especially those observed within the samples is to be made.

6. Samples will be placed in 500 ml sample jars, fixed with 10% buffered Formalin, and labeled.

7. Destructive and general algae samples are sent along with the completed external labels and appropriate Chain of Custody form to the contracted laboratory.

Note: Special certifications are required for the SCUBA work contained in this SOP. All SCUBA diving conducted under this SOP is expected to occur under a Scientific Diving Program. SCUBA divers conducting hard-bottom/ riprap destructive sampling are required to have a valid and current SCUBA certification from a recognized SCUBA certification organization (e.g., PADI, NAUI, or SSI) along with first aid, CPR/AED, and Emergency Oxygen for Scuba Diving Injuries (Divers Alert Network) certifications.

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4.0 Sample Form

BENTHIC DESTRUCTIVE

Project: _____ Station: _____ Sample: _____

Replicate	A	B	C	D	E
Sample #					
Date					
Time					
Collectors					
Azimuth					
Kicks					
Macroalgae Total % Cover					
Scraped Surface Type					
Smooth					
Irregular					
Creviced					
Finfish	0 = 0	+ = 1-10	++ = 11-100	+++ = 101+	
Cunner					
Pollock					
Other					

Comments: _____

_____

_____

_____

Figure 7. Example of benthic destructive field datasheet.

VI. Field Standard Operating Procedures for Soft-Bottom Sediment Profile Imaging (SPI; Optional)

1.0 Equipment List

The sediment profile camera system consists of a digital camera (e.g., Canon 7D, 18-megapixel sensor) enclosed in a pressure-resistant housing, a 45° prism, and a mirror that reflects an image of the sediment through the camera lens. A strobe mounted inside the prism is used to illuminate the sediment. The prism is also equipped with a video camera with a feed to the surface via cable so that prism penetration can be monitored in real time. The camera/prism system is mounted in a cradle that is secured to a larger frame, which ensures that the prism penetrates the sediment at a 90° angle. In addition, the camera frame supports a plan-view video camera mounted to view the surface of the seabed in front of the prism. Prior to every field deployment, all essential items are gathered and tested for proper operation.

2.0 Gear Deployment

A winch is used to lower the entire assembly at a steady rate to the seafloor. Images from the video-plan camera are relayed to the surface via the video cable and permit the camera operator to see the seafloor and know exactly when the camera has reached the bottom. The camera operator then can view the prism penetration and choose exactly when to record sediment profile images. Each time the camera is on the bottom, a series of 2–4 photographs is taken, generally within the first 12 seconds after bottom contact.

This sampling protocol helps to ensure that at least one usable photograph is produced during each deployment of the camera. After the required number of replicates, the camera assembly is returned to the ship. The date, time, station, replicate, water depth, and comments will be recorded in a field log. Vessel location coordinates will also be recorded with each touchdown of the camera. The digital camera saves images to compact flash solid-state memory cards. The video signal (from the plan-view video camera) is recorded on mini-DVD digital videotape for later review. The combination of video and digital images will ensure accurate and reliable collection of SPI data. The video contributes the real-time assessment component, whereas the still images provide high-resolution detail for full image analysis in the laboratory.

3.0 Collection Procedure

SPI sample collection consists of the following steps:

1) Record beginning and ending location and time of station visit
2) Record prism penetration (±1 cm), and
3) Collect 3 images at each station.

4.0 Sample Form

Coordinates at the location of each sediment profile image (SPI) sample will be entered into the field station log in Excel. A waypoint will be entered into the shipboard GPS when a sample is collected. The marker set for each waypoint will be named as the station name, with the replicate number appended. Waypoints will be stored separately for the SPI survey. A QC check of waypoints against the recorded coordinates will be done after each sample is collected by the Chief Scientist. Waypoints will be stored on the shipboard GPS until data checking confirms that all samples were collected within 37 meters of the target station location. Any sample coordinates found through data checking to be outside of the 37-meter station radius will be compared against the sample coordinates for the stored waypoint. Thus, if an incorrect waypoint is identified through data checking, the hand-entered data will be compared to the electronic waypoint on the GPS, and any error discovered in the navigational data will be corrected as necessary.

At the beginning of each survey, the date, start time, weather, and names of all sampling team members present will be entered (see Survey Log Form, Figure 4). Measurements made and samples collected will be recorded electronically. Information specific to sample collection will include:

- Station name
- Sample identification number
- Time and date of sample collection
- Sample description (color, texture, etc.)
- Samplers' initials
- Requested analyses
- Location (the latitude and longitude coordinates where a sample is collected)

VII. DATA HANDLING

1.0 Data Handling Field Datasheets

Data from sample processing that occurs in the field for all field tasks will be recorded on field datasheets. The field datasheets will be printed on an 8-1/2" x 11" sheet of weatherproof paper.

All completed datasheets will be reviewed for completeness and legibility by the originator and Chief Scientist. Field data manually recorded on field datasheets will be the responsibility of the Chief Scientist during the field activity. Datasheets will then be transferred to the Project Manager for quality control checks within 1 week of collection.

2.0 Data Handling Electronic Files

Hand-entered coordinates for sample locations will be checked against electronic waypoints on the shipboard GPS after each sample is collected. Waypoints will then be stored on the shipboard GPS until data checking confirms that all samples were collected within 37 meters of the target sampling location.

Field data recorded electronically will be the responsibility of the Chief Scientist during the field activity. Electronic water quality data and digital images files will be provided to the Project Manager within 1 week of collection.

APPENDIX B

MEP Marine Benthic Monitoring
Laboratory Standard Operating Procedures

Disclaimer: All applicable federal, state, and local laws and regulations are to be followed when conducting activities described in this Standard Operating Procedures. The Massachusetts Department of Environmental Protection and the Commonwealth of Massachusetts accept no responsibility and no liability for loss of any kind, including personal injury or property damage due to the work and/or activities described in this Laboratory Standard Operating Procedures.

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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 dissolved oxygen (DO)) to highly stressed (high organic matter, low DO). 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 2020). 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).

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 (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 analysis 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 Sediments
    - Grain size
    - 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.

US EPA (U.S. Environmental Protection Agency). 2020. National Coastal Condition Assessment 2020 Field Operations Manual. April 2020. EPA- 841-F-19-005. U.S. Environmental Protection Agency, Office of Water, Office of Wetlands, Oceans and Watersheds. Washington, DC. 149 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) 2020 Laboratory Operations Manual (US EPA 2021). For additional program details refer to the MEP Benthic Monitoring QAPP.

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)

a. 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 COC 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 COC 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. Record the sample collection information on a Sample Card or datasheet.
2. Carefully decant the reagent alcohol from the sample container by pouring the fluid through a 0.5 mm or 1.0 mm sieve (selected based on the sample type, benthic infaunal or hard-bottom/riprap destructive) into a separate container. Gently rinse the sample with tap water using the spray nozzle, being careful to use a low-volume spray so the organisms will not be damaged. Rinse sample until residual water coming through the sieve is clean. Using the spray, carefully direct the sample contents into a clean beaker. Ensure that all organisms have been removed from the sieve.
3. Remove sieved organisms from the beaker and place into a sorting tray. Ensure all organisms have been removed from the beaker.
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 a vial with reagent alcohol 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 10.0.
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 Sample Preparation - Subsampling

During the sorting process, if a sample contains organisms that are not separable from the sediment, then an additional step of subsampling may be warranted. For example, in the Pilot Study: Pleasant Bay (Sweeny and Rutecki 2019), an extremely large number of very small juvenile bivalves were essentially the same size as the sieve (0.5 mm) and therefore could not be practicably counted. The approved solution was to subsample the material that remained on the 0.5 mm mesh.

Note: No subsampling will occur without prior discussion and approval of the project manager, MassDEP, and/or the Town/client.

For samples that have been approved for subsampling use the following steps:

1. Remove the lid from the sample container and remove the internal sample label. Record the sample collection information on a Sample Card or datasheet.
2. Carefully decant the reagent alcohol from the sample container by pouring the fluid through a through a 0.5 mm or 1.0 mm sieve (selected based on the sample type) into a separate container.
3. Following removal of the preservative, gently wash the sample and circulate (elutriate) sample with water and allow any light material and organisms that might be in the gravel/sand to float to the top of the water. Pour the water through the sieve and set material aside for sorting. Sort as above.
4. Repeat Step 3 until the water runs clear.
5. To separate the heavy material into homogeneous sizes to facilitate sorting, sieve the remaining material through a stack of different sized sieves finishing with a 0.5 mm or 1.0 mm sieve at the bottom of the stack. Material on the different sized sieves that does not require subsampling will be set aside for sorting and sorted as above.

6. Place the material that is to be subsampled into a pan (for example 30.5 cm by 30.5 cm [12 in by 12 in] inside dimensions with marks at the edges for grids 6 cm by 6 cm) marked with a grid. Spread the sample material over the bottom of the pan as evenly as possible. Move the sample into the corners of the pan using forceps, spoon, or by hand. Vibrate or shake the pan gently to help spread the sample.
7. Use a random number generator to select at least 10% (or the approved percentage) of the squares to ensure that the subsample material is representative of the overall sample. For example, if the tray is subdivided into 30 squares, then 3 squares would be selected for sorting.
8. Remove all the material from the selected squares. This can be done by placing a metal dividing frame over the sample at the approximate location of the square selected for processing. Use a pair of rulers or other straight edges to facilitate lining up the frame at the intersection, if necessary. Remove the material within the frame using a scoop, teaspoon, forceps, or dropper depending on the consistency of the sample.
9. Inspect the tray for any remaining organisms. Use the following rules when dealing with organisms that lie on the line between two squares:
    a. An organism belongs to the square containing its head.
    b. If it is not possible to determine the location of the head (i.e., for polychaetes), the organism is considered in the square containing most of its body.
    c. If the head of an organism lies on the line between two squares, all organisms on the top of a square and those on the right side of a square belong in that square, and are picked with that square.
10. Place the subsampled material into a sorting tray and proceed with sorting as above.
11. Record the number of organisms found in subsampled material on the Sample Card or datasheet. Record the squares selected and the total number of squares samples on the Sample Card or datasheet. If laboratory protocol is to record total for subsample material on cards or datasheets, multiple raw counts by appropriate multiplier and enter total number of organisms
12. Remove the remaining material left on the sorting pan from the selected squares (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.
13. Return all material not subsampled (remaining on the tray) to the original container with the preservative that was set aside. This container will include the original sample labels. Prepare two additional labels “Unsorted Sample Remains” and place one inside the container and attach the other to the outside of the container. Replace the lid and tighten securely. Archive the container until all appropriate QC checks are completed (subsampling and taxonomy), and in accordance with survey and laboratory requirements.

In the Pilot Study: Pleasant Bay (Sweeny and Rutecki 2019) example from above the following procedure was used. The samples that had considerable quantities of sand containing large numbers of the very small bivalves (*Gemma gemma*) were subsampled to facilitate the counting effort and estimate the number of *G. gemma*. The project manager and benthic taxonomic experts determined that 25% of the material retained on the 0.5 mm sieve the would be selected for sorting. This was a conservative approach to ensure a valid estimate of the small bivalves. MassDEP was notified of the issue and approved the subsampling solution. Following removal of the preservative, samples were gently washed, and the light material was elutriated from the matrix and set aside for sorting. To separate the heavy material into homogeneous sizes to facilitate sorting, the remaining material was sieved through a stack of different sized sieves finishing with a 0.5 mm sieve at the bottom of the stack. The sand with the juvenile bivalves retained on a 0.5 mm mesh was spread out evenly in a pan marked off with 36 squares. To sort ¼ (25%) of the sand, a random numbers table was used to select 9 squares of material out of the total of 36 squares. Material from the 9 squares was removed from the pan and all *G. gemma*, as well as any incidental remaining molluscs were counted. Raw counts were multiplied by 4 to achieve the estimate for the entire sample and entered on the datasheet.

6.0 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 COC 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, which are 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 the World Register of Marine Species (WORMS) website to check the scientific name to ensure that there have not been any name changes: http://www.marinespecies.org/aphia.php?p=search
    b. It should be noted if the target taxonomic level cannot be achieved due to immature or damaged organisms.
    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. Save one specimen from each taxa in a labeled vial with 70% reagent alcohol for the reference collection. The label should include the lowest possible taxonomic level of the organism, embayment collected from, station collected from, and date of collection. 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.

7.0 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 datasheets, 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.

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, a qualified peer, and supervisory and/or QA personnel.

Table 41. Benthic macrofaunal and destructive sample data codes. Data codes are revised from NCCA 2020 (US EPA 2021).

Field	Format	Description
EVENT_ID	Character	Identifier of sampling event (survey)
LAB_NAME	Character	Name of lab
DATE_RECEIVED	MM/DD/YY HH:MM	Date sample was received by lab
SITE_ID	Character	Site identification code as used on sample label
SAMPLE_ID	Character	Sample ID as used on field sheet (on sample label)
SAMPLE_TYPE	Character	INFAUNAL or DESTRUCTIVE
DATE_COLLECTED	MM/DD/YY HH:MM	Date sample was taken

Table 1. Continued.

Field	Format	Description	
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 42. Benthic macrofaunal taxonomic identification data codes. Data codes are revised from NCCA 2020 (US EPA 2021).

Field	Format	Description
EVENT_ID	Character	Identifier of sampling event (survey)
LAB_NAME	Character	Name of lab
DATE_RECEIVED	MM/DD/YY HH:MM	Date sample was received by lab
SITE_ID	Character	Site identification code as used on sample label
SAMPLE_ID	Character	Sample ID as used on field sheet (on sample label)
DATE_COLLECTED	MM/DD/YY HH:MM	Date sample was taken
DATE_TAXON	MM/DD/YY	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
APHIA_ID	Numeric	World Register of Marine Resources (WoRMS) Database ID. 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)

Table 2. Continued.

Field	Format	Description	
CITATION	Character	Citation for reference used to identify organism, if taxon not present in WoRMS.	
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
		IM	Immature
		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	

8.0 Sample and Record Retention

The laboratory shall retain:

1. Macrofauna samples (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.

9.0 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.

- **9.1 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 ≤ 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 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.

- **9.2 Identification and Enumeration**

The same basic QC principles described in Section 9.1 (Sorting) will generally 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 Section 9.1. 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.

10.0 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 11.

11.0 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.

12.0 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.

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Bousfield, E.L. 1973. Shallow-water Gammaridean Amphipoda of New England. Comstock Publishing Associates, Ithica, NY.

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Sweeny, M. and D. Rutecki. 2019. Embayment-Specific Study Plan: Pleasant Bay Pilot Field Study for MassDEP Massachusetts Estuaries Project. Prepared by Normandeau Associates, Inc., Falmouth, MA. 45 pp.

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Chain of Custody Form

Project Name: _____
Project Number: _____
Originating Contact: _____
Originator Location: _____
Final Destination: _____
Sampler(s): _____

Sample		Collection		Containers		Grab.	Comp.	Presv.		Parameters				Page of
No.	Identification	Date	Time	No.	Type									Method of Shipment:
														Comments Below:
				Total										

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:
Date:	Date:	Date:	Date:	Date:	Date:

Figure 8. Example of a Chain of Custody Form.

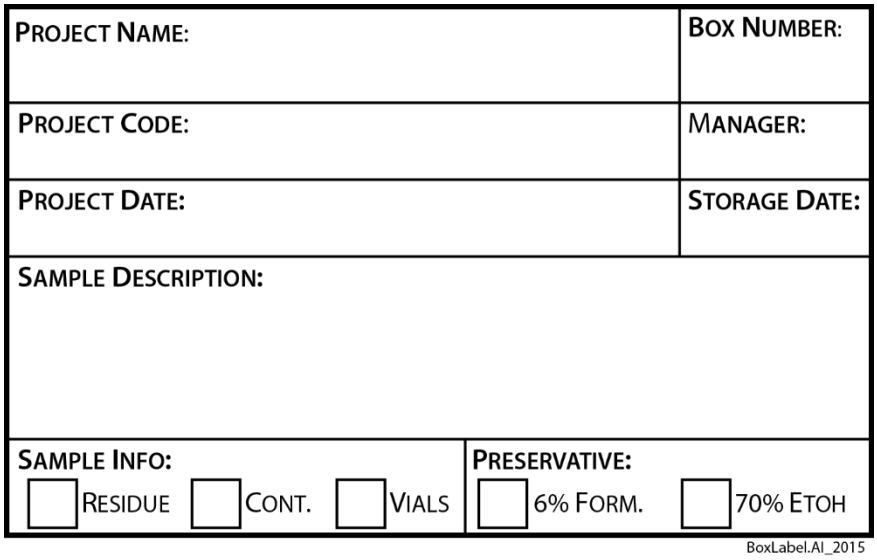

Figure 9. Example of a Storage Label.

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 10. 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 at 4 °C. The holding time for grain size analysis and TOC is 28 days. For additional program details refer to the MEP Benthic Monitoring QAPP.

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 manufacturer'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 COC form in the shipment.

b. Notify the Project Manager if any jars were broken and/or there are discrepancies between the COC form and jars.

3. Maintain the COC 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 crew ships 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 have 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 43. Sediment analysis sample data codes. Data codes are revised from NCCA 2020 (US EPA 2021).

Field	Format	Description	
EVENT_ID	Character	Identifier of sampling event (survey)	
STAT_ID	Character	Station identification code as used on sample label	
SAMPLE_ID	Character	Sample ID as used on chain of custody form (on sample label)	
DATE_COLLECTED	MM/DD/YY HH:MM	Date sample that the field crew collected the sample	
LAB_NAME	Character	Name of lab	
DATE_RECEIVED	MM/DD/YY	Date sample was received by lab	
ANALYSIS_TYPE	Numeric	GRAIN SIZE, TOC, or Contaminant	
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 US EPA Method 9060. Results will be reported in units of mg/kg or % with a method detection limit (MDL) of 0.01% and a target accuracy of 10% (US EPA 2021).

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.

4.2.1 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 5gallon receptacle bucket, on which nested sieves will snuggly fit
- Sink with a spray nozzle
- Approximately 4 feet of rubber tubing
- Disposable tin foil and paper cupcake tins/baking cups or small similarly sized metal pans (any metal that can be heated to 135 °F)
- Drying oven (135 °F)
- Electronic digital scale (0.01 g accuracy)
- 500 ml squirt bottle filled with tap water
- Spoon or spatula

4.2.2 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 ¼ of the sample) can be placed into the top sieve and rinsed until the water has passed through all sieves, then another ¼ 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 μ 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 datasheet 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 μ (0.25 to < 0.5 mm)
- Fine sand = 125 μ to < 250 μ (0.125 to < 0.25 mm)
- Very fine sand = 63 μ to < 125 μ (0.0625 to < 0.125 mm)
- Silt = < 63 μ (<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, 4 and 5 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 44. Analysis data codes for TOC and other parameters including contaminants with single result values. Data codes are revised from NCCA 2020 (US EPA 2021).

Field	Format	Description	
YEAR	Numeric	Survey year	
EVENT_ID	Character	Identifier of sampling event (survey)	
STAT_ID	Character	Station identification code as used on chain of custody form (on sample label)	
SAMPLE_ID	Character	Sample ID as used on chain of custody form (on sample label)	
SAMPLE_NUMBER	Character	Sample number as used on chain of custody form (on sample label)	
DATE_COLLECTED	MM/DD/YY HH:MM	Date sample that the field crew collected the sample	
LAB_NAME	Character	Name of lab	
DATE_RECEIVED	MM/DD/YY	Date sample was received by lab	
ANALYSIS_TYPE	Character	TOC or Contaminant	
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	

Table 4. Continued.

Field	Format	Description
METHOD	Character	Laboratory method used
DATE_ANALYZED	MM/DD/YY	Date that the analysis started
HOLDING_TIME	Y/N	Analysis performed within holding time
MDL	Numeric	Lab method detection limit
RL	Numeric	Actual Reporting limit (based on the unique matrix of sediment for each batch of samples)
MOISTURE	Numeric	Moisture in the sample (value used by lab to convert wet units to dry)
MOIST_UNIT	Character	Unit used to report moisture (% or mg/kg)
DILUTION	Numeric	Dilution of sample (blank if no dilution)
RESULT	Numeric	Concentration value
RESULT_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

Table 45. Sediment grain size analysis data codes. Data codes are revised from NCCA 2020 (US EPA 2021).

Field	Format	Description
YEAR	Numeric	Survey year
EVENT_ID	Character	Identifier of sampling event (survey)
STAT_ID	Character	Station identification code
SAMPLE_ID	Character	Sample ID as used on chain of custody form (on sample label)
SAMPLE_NUMBER	Character	Sample number as used on chain of custody form (on sample label)
DATE_COLLECTED	MM/DD/YY HH:MM	Date sample that the field crew collected the sample
ANALYSIS_TYPE	Numeric	GRAIN SIZE

CONDITION_CODE	Character	Condition codes describing the condition of the sample upon arrival at the laboratory.

Table 5. Continued.

Field	Format	Description	
		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	MM/DD/YY	Date that the analysis started	
HOLDING_TIME	Y/N	Analysis performed within holding time	
MDL	Numeric	Lab method detection limit	
RL	Numeric	Actual Reporting limit (based on the unique matrix of sediment for each batch of samples)	
MOISTURE	Numeric	Moisture in the sample (value used by lab to convert wet units to dry)	
MOIST_UNIT	Character	Unit used to report moisture (% or mg/kg)	
DILUTION	Numeric	Dilution of sample (blank if no dilution)	
PCT_Gravel	Numeric	Percentage of gravel	
PCT_VC_Sand	Numeric	Percentage of very coarse sand	
PCT_C_Sand	Numeric	Percentage of coarse sand	
PCT_M_Sand	Numeric	Percentage of medium sand	
PCT_F_Sand	Numeric	Percentage of fine sand	
PCT_VF_Sand	Numeric	Percentage of very fine sand	
PCT_Silt	Numeric	Percentage of silt	
PCT_Clay	Numeric	Percentage of clay	
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 of the analysis

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.

US EPA (U.S. Environmental Protection Agency). 2021. National Coastal Condition Assessment 2020 Laboratory Operations Manual. Version 1.2 March 2021. EPA- 841-F-19-004. U.S. Environmental Protection Agency, Office of Water. Washington, DC. 169 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.

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 (COC) 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 square meter 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 Coastal and Marine Ecological Classification Standard (CMECS) sediment grain size descriptors (Table 6). Additional substrates that could be observed in digital images are presented in Table 7. Habitat relief, the difference in elevation between two surfaces, will be characterized as none, low, moderate, and high as described in Table 8. 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 9. 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 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. The data from the video will initially be entered either on datasheets 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 46. 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 47. 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).
Crepidula Reef Substrate	Shell Reef that is primarily composed of conglomerated *Crepidula* 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 48. Habitat relief descriptions.

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

Table 49. 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 50. 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 CMECS for sediment grain size (Table 6). Additional substrates that could be observed in the digital images are described in Table 7. Habitat relief, defined as 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 8). 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 9). 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 11. 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 reviewed[10] 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 datasheets and then into an Excel spreadsheet. The spreadsheet will be delivered to the Project Manager.

Table 51. 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 datasheets, 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. The underwater digital image codes are presented in Tables 12 and 13.

[10] 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.

Table 52. Underwater digital image survey codes.

Field	Format	Description
EVENT_ID	Character	Identifier of sampling event (survey)
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 identification code
STAT_ARRIV_ LOCAL	MM/DD/YY HH:MM	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 53. Underwater digital image analysis data codes.

Field	Format	Description	
EVENT_ID	Character	Identifier of sampling event (survey)	
LAB_NAME	Character	Name of lab	
STAT_ID	Character	Station identification code	
DATE_COLLECTED	MM/DD/YY	Date image was taken	
STAT_ARRIV_LOCAL	MM/DD/YY HH:MM	Station arrival date and time (local time)	
IMAGE_DATE_TIME_BEG_LOCAL	MM/DD/YY HH:MM	Video only: Time of the beginning of this video (local time)	
IMAGE_DATE_TIME_END_LOCAL	MM/DD/YY HH:MM	Video only: Time of the end of this video (local time)	
USABLE_MINUTES	Numeric	Video only: Number of usable minutes between the image_date_time_beg and image_date_time_end	
DEPTH_BEG	Numeric	Video only: Depth of water at image_time_beg (meters)	
DEPTH_END	Numeric	Video only: Depth of water in at image_time_end (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
		rr	Riprap
		s	Sand
RELIEF_CODE	Character	Codes describing the habitat relief observed in the digital image	
		Code	Definition (See Table 8)
		n	None
		l	Low
		m	Moderate
		h	High

Table 13. Continued.

Field	Format	Description	
SED_DRAPE_CODE	Character	Codes describing the sediment drape observed in the digital image	
		Code	Definition (See Table 9)
		a	Absent
		l	Low
		m	Moderate
		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 10 and 11)
		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.

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.

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 6)
- 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 14)
- 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 15 and discussed in more detail in Appendix D of the MEP Benthic Monitoring QAPP. 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 16 and 17 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 54. 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 55. 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	—	V	Count
(Oxic) Voids	Number	V	Count
• Successional Stage	—	V	Identify
Organism-Sediment Index (OSI)	—	CA	Derived from aRPD, Successional Stage, and Voids (Rhoads and Germano 1982, 1986)

[1] V: Visual measurement or estimate
CA: Computer analysis

Table 56. Sediment profile imaging survey data codes.

Field	Format	Description
EVENT_ID	Character	Identifier of sampling event (survey)
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	MM/DD/YY HH:MM	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 57. Sediment profile imaging analysis parameter codes.

Field	Format	Unit	Description
EVENT_ID	Character		Identifier of sampling event (survey)
LAB_NAME	Character		Name of lab
STAT_ID	Character		Station identification code
DATE_COLLECTED	MM/DD/YY HH:MM		Date image was taken
DATE_ANALYSIS	MM/DD/YY		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 will be analyzed, (2) all parameters defined in this SOP and in the Benthic Monitoring QAPP will be

analyzed for all images, and (3) that analytical systems used will 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.

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APPENDIX C

Quality Assurance Project Plan Agreement Form

QUALITY ASSURANCE PROJECT PLAN (QAPP) AGREEMENT FORM FOR

Embayment:_____
(Select complete or partial implementation below)

We, the undersigned, have read and understand the requirements outlined in the QAPP for the Massachusetts Estuaries Program Benthic Monitoring, and establish that this embayment study will meet the overall intent and requirements set forth in the ***entire*** QAPP.

Project Manager		**Program Quality Assurance (QA) Officer**	
_____		_____	
Name	Date	Name	Date
Address		Address	
Phone:	Email:	Phone:	Email:

We, the undersigned, have read and understand the requirements outlined in the QAPP for the Massachusetts Estuaries Program Benthic Monitoring, and establish that this embayment study will ***partially*** meet the requirements set forth in the QAPP. We will be following the requirements set forth in Sections _____.

Project Manager		**Program Quality Assurance (QA) Officer**	
_____		_____	
Name	Date	Name	Date
Address		Address	
Phone:	Email:	Phone:	Email:

MassDEP acknowledges that the above signed have agreed to completely or partially follow the Massachusetts Estuaries Program Benthic Monitoring QAPP for the above named embayment study.

_____	_____
Matthew Reardon, MEP Contact, MassDEP	Sue Flint, QA Officer, MassDEP
Date	Date
8 New Bond Street, Worcester, MA 01606	8 New Bond Street, Worcester, MA 01606
Phone: 508-849-4002 Fax: 508-791-4131	Phone: 508-767-2789 Fax: 508-791-4131
Email: matthew.reardon@mass.gov	Email: suzanne.flint@mass.gov

APPENDIX D

Sediment Profile Image Parameters

The following paragraphs describe the parameters measured from the sediment profile images.

Sediment grain size is a geotechnical feature of the sediments that is used to determine the type of sediments present. The nature of the physical forces acting on a habitat can be inferred from grain-size distribution of the sediments. The sediment type descriptors used follow the Wentworth (1922) standard as described in Folk (1974) and the CMCES (FGDC 2012) and represent the major modal class for each layer identified in an image (Table D-1). Sediment grain size is determined by comparing the collected images with a set of standardized images taken of sediments for which mean grain size has been determined by laboratory analyses. Sediment grain sizes ranging from pebble/rock to gravel, to sand, to silt, and clay can be estimated accurately from the images.

Table D-1. 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

Prism penetration provides a geotechnical estimate of sediment compaction, with the profile camera prism acting as a dead-weight penetrometer. The farther the prism enters into the sediment, the softer the sediment and likely the higher the water content. Penetration is measured simply as the distance the sediment moves up the 25-cm length of the faceplate. If the weight of the camera frame is not changed during field image collection, the prism penetration provides a means for assessing the relative sediment compaction between stations or different habitat types.

Surface relief is measured as the difference between the maximum and minimum distance the prism penetrates. This parameter provides an estimate of small-scale bed roughness, on the order of the prism faceplate width (15 cm). The causes of roughness often can be determined

from a visual analysis of the images. In physically dominated sandy habitats, surface relief typically consists of small sand waves or bed forms. In muddy habitats, surface relief is typically irregular (being primarily derived from biological activity of benthic organisms, which form mounds or pit during feeding and burrowing) or smooth. Biological surface roughness can range from small fecal mounds and tubes to large colonies of hydroids or submerged aquatic vegetation (SAV). Surface relief provides qualitative and quantitative data on habitat characteristics, which can be used to evaluate recent and existing habitat quality.

Apparent redox potential discontinuity (aRPD) layer is an important estimator of benthic habitat quality. It is the depth to which sediments are oxidized. The term apparent is used in describing this parameter because no actual measurement is made of the redox potential. An assumption is made that, given the complexities of iron and sulfate reduction-oxidation chemistry, reddish-brown sediment color tones are indications that the sediments are oxic (oxidized), or at least are not intensely reducing (Diaz and Schaffner 1988). This is in accordance with the classical concept of RPD depth, which associates it with sediment color (Fenchel 1969).

The depth of the aRPD is defined as the area of all the pixels in the image discerned as being oxidized divided by the width of the digitized image. The area of the image with oxic sediment is obtained by digitally manipulating the image to enhance characteristics associated with oxic sediment (greenish-brown color tones). The enhanced area then is determined from a density slice of the image or, if image quality is poor, the area is delineated with the cursor.

The aRPD is very useful in assessing the quality of a habitat for epifauna and infauna from physical and biological perspectives. Rhoads and Germano (1986), Day et al. (1988), and Diaz and Schaffner (1988) found the depth of the RPD from profile images to be directly correlated to the quality of the benthic habitat in polyhaline and mesohaline estuarine zones. Thin RPDs, on the order of a few millimeters, tend to be associated with some environmental stress, whereas areas with deep RPDs, that is, deeper than 3 cm, usually were found to have flourishing epibenthic and infaunal communities.

The aRPD modifiers used follow the CMCES (FGDC 2012) and are presented in Table D-2.

Table D-2. 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

Surface features include a variety of physical and biological features that can be seen at or on the sediment surface. These can range from submerged aquatic vegetation (SAV), worm tubes, fecal

pellets, epibenthic organisms, bacterial mats, algal mats, shells, mud clasts, and bed forms to feeding pits and mounds. Each feature provides information on the type of habitat and its quality. Certain surface features are indicative of the overall nature of a habitat. For example, bedforms are always associated with physically dominated habitats, whereas worm tubes or feeding pits are indicative of a more biologically accommodated habitat (Rhoads and Germano 1986; Diaz and Schaffner 1988). Surface features are visually evaluated from each slide and compiled by type and frequency of occurrence.

Subsurface features include a variety of features such as burrows, water-filled voids, SAV rhizomes, infaunal organisms, gas voids, shell debris, detrital layers, and sediment lenses of different grain size. Subsurface features also reveal a great deal about the physical-biological control occurring in a habitat. For example, the presence of gas voids with a mixture of nitrogen and methane from bacterial metabolism (Reineck and Singh 1975) has been found to be an indication of anaerobic metabolism (Rhoads and Germano 1986) and associated with high rates of bacterial activity. Muddy habitats with large amounts of methane gas are generally associated with areas of oxygen stress or high organic loading (Day et al., 1988). On the other hand, habitats with burrows, infaunal feeding voids, and/or visible infauna are generally more biologically accommodated and considered unstressed.

Successional stages of the fauna in a habitat can be estimated by using SPI data (Rhoads and Germano 1982, 1986, Nilsson and Rosenburg 2000). The CMECS describes four modifiers for community stage in soft-bottom areas (FGDC 2012; Figure D-1, Table D-3). For evaluating SPI, the CMECS recommends following the guidelines from Rhoads and Germano (1982, 1986) used to determine Organism-Sediment Index (OSI), or using the Nilsson-Rosenberg Benthic Habitat Quality (BHQ) metric (Nilsson and Rosenberg 1997, 2000, FGDC 2012). Stage I communities have characteristics that are associated with pioneering or colonizing assemblages, such as dense aggregations of small polychaete tubes at the surface and shallow apparent RPD layers. Stage III communities are advanced or near equilibrium and show characteristics such as deep apparent RPD layers and subsurface feeding voids. Stage II is intermediate or transitional assemblage from Stages I and III, and has characteristics of both (Rhoads and Germano 1986).

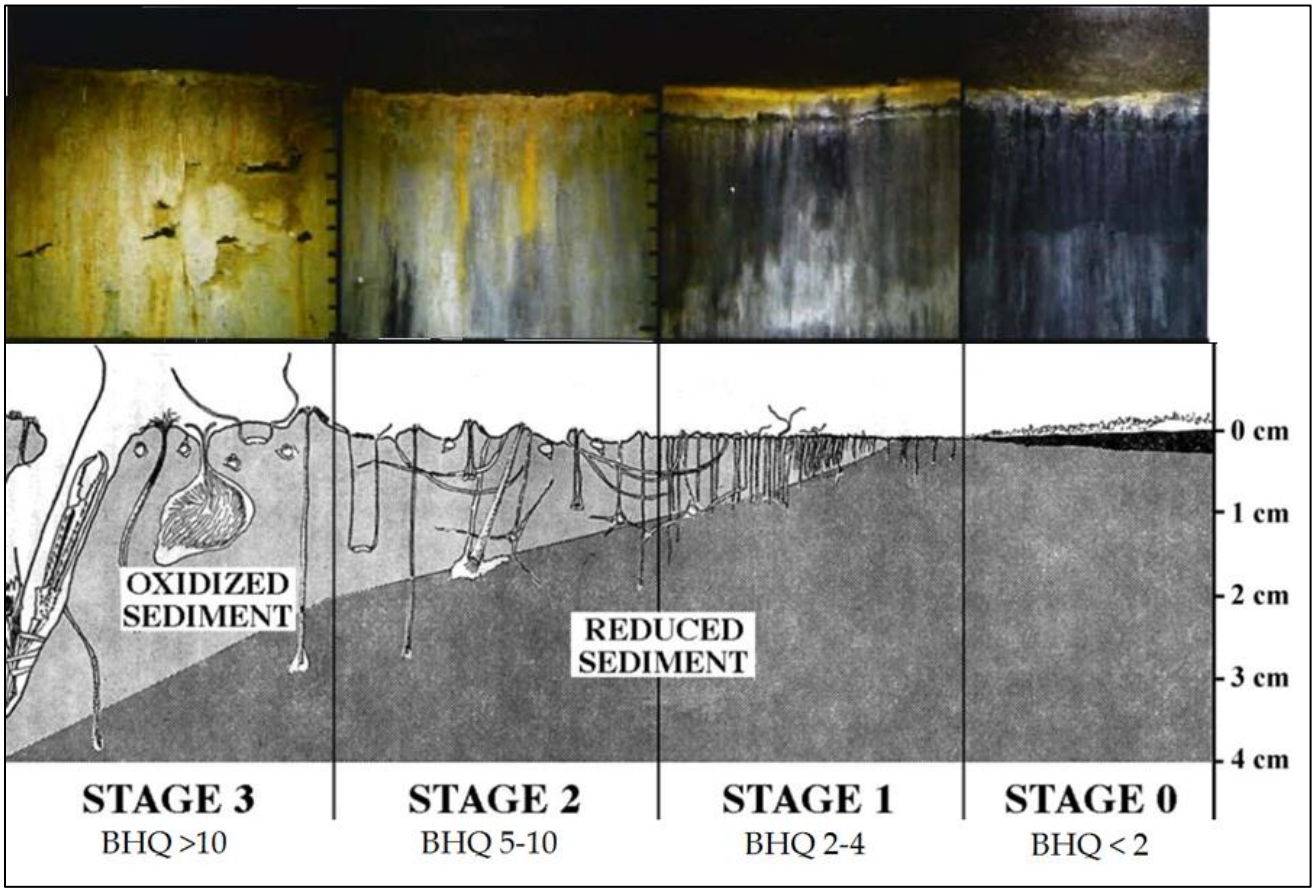

Figure D-1. Benthic community stages in soft-bottom areas along a gradient of increasing environmental disturbance from left to right with associated benthic habitat quality (BHQ) index values shown (modified from Nilsson and Rosenburg 2000 and FGDC 2012).

Table D-3. Community successional stages (from FGDC 2012).

Community Successional Stage	Description
Stage 3	These communities are identified by larger, long-lived, deep burrowing fauna or by evidence of the activities of those fauna; burrowing activities typically extend deeper than 5 centimeters. Characteristic species vary among localities and among environments; species can be identified through appropriate regional literature. Common surface expression may include very large tube-building fauna (> 3 millimeters in diameter or > 30 mm in length), larger fecal mounds, burrowing urchins or ophiuroids, pits or tunnel openings, or large digging spoils associated with pits or tunnels. Subsurface characteristics include oxygenated or active faunal feeding voids at 5 centimeters or deeper, active tunnels (subsurface excavations with a lumen width of > 1 centimeter) at depth, or presence of large polychaetes or other fauna. Frequently, evidence of smaller, opportunistic fauna will also be present in Stage 3 communities. Extensive bioturbation will be evidenced by deep RPD and aRPD depths. Stage 3 will have Nilsson-Rosenberg Benthic Habitat Quality (BHQ) metric > 10.
Stage 2	Communities are characterized by fauna of intermediate sizes typically inhabiting the upper 2–4 centimeters of sediment. This stage is considered transitional and is often variable; in a percentage of samples it will be difficult to clearly distinguish Stage 2 from other stages. Regional literature identifying species typical of Stage 2 may be referenced. Surface evidence of Stage 2 communities includes openings to small burrows (defined as excavations with a lumen width < 1 centimeter) and the presence of mid-sized tube dwelling fauna (e.g., robust Ampelisca tube mats; tubes > 2 millimeters in diameter; or tubes longer than 30 millimeters if very thin). Subsurface evidence includes burrows of polychaetes or other fauna in the upper 2–4 centimeters of sediment, small shallow-dwelling opportunistic bivalves, and small feeding voids in the upper 4 centimeters of sediment. Stage 2 will have BHQ values that range from 5 to 10.
Stage 1	Communities are inhabited by small opportunistic fauna (e.g., capitellids and spionids) in the upper centimeter of sediment. Larger fauna are not present, although juvenile individuals of larger species may occur. Names of small, opportunistic local species typical of Stage 1 are available in the regional literature. Surface expressions include small tubes (< 2 millimeters in diameter) of polychaetes or other fauna, or evidence of oligochaete burrowing activities. Subsurface evidence of either small worms or small burrow structures will primarily occur in the upper centimeter of sediment. Bioturbation depths will be shallow, with an aRPD depth typically > 2 millimeters to < 2 centimeters. Stage 1 will have BHQ values that range from 2 to < 5.
Stage 0	These oligozoic soft-sediment areas show little evidence of multi-cellular life. Benthic grab samples that are retrieved and processed under magnification from Stage 0 areas will generally produce low numbers of small macrofauna or meiofauna. Multicellular fauna will not be obvious to the unassisted eye when examining sediment, and it will not be obvious in high-resolution seafloor images. Bacterial mats may be present. No evidence of active bioturbation exists, and aRPD depths are typically < 2 millimeters. Stage 0 communities will have BHQ values that range from 0 to 1.99.

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