# Marine Benthic Macrofaunal Monitoring Guidance to Support TMDLs and Habitat Condition Assessments

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# Acronyms and Abbreviations

| | |
|---|---|
| AMBI | AZTIs Marine Biotic Index |
| APBI | Acadian Province Benthic Index |
| AUV | Autonomous Underwater Vehicle |
| BEQ | Benthic Environmental Quality |
| B-IBI | Benthic Index of Biotic Integrity |
| BRI | Benthic Response Index |
| CMECS | Coastal and Marine Ecological Classification Standard |
| CWMP | Comprehensive Wastewater Management Plan |
| CZM | Massachusetts Office of Coastal Zone Management |
| DDT | Dichloro-Diphenyl-Trichloroethane |
| DMF | Massachusetts Division of Marine Fisheries |
| EMAP | Environmental Monitoring and Assessment Program |
| EPA | U.S. Environmental Protection Agency |
| GPS | Global Positioning System |
| GRTS | Generalized Random Tessellation Stratified |
| HD | High-definition |
| IBI | Index of Biotic Integrity |
| M-AMBI | Multivariate AZTIs Marine Biotic Index |
| MAIA | Mid-Atlantic Integrated Assessment Program |
| MassDEP | Massachusetts Department of Environmental Protection |
| MDS | Multidimensional Scaling |
| MEP | Massachusetts Estuaries Project |
| MLOE | Multiple Lines of Evidence |
| MWP | Mussel Watch Project |
| MWRA | Massachusetts Water Resources Authority |
| NCCA | National Coastal Condition Assessment |
| NPDES | National Pollutant Discharge Elimination System |
| NS&T | National Status and Trends |
| OSI | Organism Sediment Index |
| PAH | Polycyclic Aromatic Hydrocarbon |
| PBDE | Polybrominated Diphenyl Ethers |
| PCB | PolyChlorinated Biphenyls |

| | |
|---|---|
| QAPP | Quality Assurance Project Plan |
| RBI | Relative Benthic Index |
| ROV | Remotely Operated Vehicle |
| RPD | Redox Potential Discontinuity |
| SCCWRP | Southern California Coastal Water Research Project |
| SEABOSS | SEABed Observation and Sampling System |
| SEIS | Supplemental Environmental Impact Study |
| SOP | Standard Operating Procedure |
| SPI | Sediment Profile Image |
| SQO BLOE | Sediment Quality Objectives Benthic Line of Evidence |
| TMDL | Total Maximum Daily Load |
| TOC | Total Organic Carbon |
| TON | Total Organic Nitrogen |
| TTD | Total Taxonomic Distinctness |
| USGS | United States Geological Survey |
| US-M-AMBI | Multivariate AZTIs Marine Biotic Index Developed for US Coastal Waters |
| VPBI | Virginian Province Benthic Index |

# 1 Background

The Massachusetts Department of Environmental Protection (MassDEP) established the Massachusetts Estuaries Project (MEP) to monitor and protect estuarine ecosystems in southeastern Massachusetts embayments. The regulatory basis for the MEP is provided by the "Massachusetts Surface Water Quality Standards" (314 CMR 4.00), which implement provisions of the federal Clean Water Act. The Water Quality Standards provide criteria for the protection of surface waters in coastal Massachusetts embayments. Although the Water Quality Standards were designed to control eutrophication by regulating both point and non-point nutrient discharges, they do not include specific thresholds for nitrogen. The strategy of the MEP was to develop site-specific nitrogen thresholds based on existing conditions, and then to calibrate and refine those thresholds using the results of follow-up monitoring for selected water quality indicators.

Howes et al. (2003) described the goals and approach of the MEP 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. Howes et al. (2003) also described the types of indicators that were selected to assess water quality, including dissolved oxygen levels, ecological diversity, and the presence of certain animal and plant species. The water quality indicators 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 Management Model Approach being used by the MEP (Howes et al. 2003).

Since the start of the MEP in 2001, approximately 70 estuaries in southeastern Massachusetts have been assessed. The technical reports for these estuaries document baseline water quality and identify the actions required to restore nutrient (total nitrogen) impaired waters. Waters that are found to be impaired are listed on the MassDEP 303(d) list and the state is required to develop plans to restore water quality. Total Maximum Daily Loads (TMDLs) for Total Nitrogen have been established for each estuary assessed. Many communities have begun the process of integrated water resources management planning or completed preparation of Comprehensive Water Resources Management Plans (CWRMPs) or Watershed Management Plans (WMPs) and have committed to on-going water quality monitoring programs. Additionally, the MassDEP has committed to eelgrass monitoring in coastal estuaries in 3-year periods as part of MassDEP's Wetlands Conservancy Program.

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. MassDEP is also interested in developing a tiered approach to conducting benthic surveys that would provide reliable information that is comparable to prior data collection efforts to ensure that judgments can be made between pre- and post- TMDLs conditions while keeping costs to and efforts by communities low. The goal of this review is to develop guidelines that can be used by parties outside MassDEP to collect benthic data that will be of sufficient quality to be used in management decisions.

# 2 Methodology Used to Review and Update Benthic Macrofauna Monitoring

Sampling methods and approaches for conducting benthic infaunal monitoring were reviewed from the existing MEP guidance documents, the MEP Technical Reports for the Cape Cod and South Coast/Buzzards Bay regions, existing literature for other federal, state, and regional benthic monitoring programs, and published literature.

Normandeau reviewed the following MEP guidance documents for the existing program: Howes et al. (2003), the MEP Quality Assurance Project Plan (QAPP; Howes and Samimy 2003), UMass Dartmouth (2004), and Howes and Samimy (2005). These documents contain the methods that have been used to assess the benthic habitat since the start of the MEP. Thirty-three embayment reports for the Cape Cod and South Coast/Buzzard Bay regions were reviewed for the benthic assessment methods, site specific method deviations, and any method modifications (Table 1). The Pleasant Bay System (Howes et al. 2006a), West Falmouth Harbor System (Howes et al. 2006b), and Wellfleet Harbor Embayment System (Howes et al. 2017a) reports were given priority based on MassDEP's interest in developing a pilot field program to test the approach, procedures, and sampling methods developed and/or recommended during this review process. The Pleasant Bay and West Falmouth Harbor estuarine systems were selected as they have been previously characterized, have established TMDLs, and the surrounding Towns have implemented measures to reduce nitrogen loads. The Wellfleet Harbor System was selected as a reference embayment since it has been previously assessed and is relatively unimpaired.

The literature for other existing federal, state, and regional benthic monitoring programs was reviewed for approaches and methodology. The programs reviewed included: the U.S. Environmental Protection Agency (EPA) National Coastal Condition Assessment (NCCA), the National Oceanic and Atmospheric Administration (NOAA) National Status and Trends Program, the Massachusetts Office of Coastal Zone Management Seafloor and Habitat Mapping Program, the Massachusetts Water Resources Authority (MWRA) Benthic Monitoring Program and the Southern California Bight Regional Monitoring Program.

**Table 1. List of Massachusetts Estuaries Project embayment technical reports for the Cape and South Coast/Buzzard Bay regions that were reviewed.**

| Embayment Systems | Citation |
|---|---|
| **Region: Cape Cod** | |
| Allen, Wychmere, and Saquatucket Harbor Embayment System | Howes et al. 2010a |
| Barnstable Great Marshes-Bass Hole Estuarine System | Howes et al. 2017b |
| Bass River Embayment System | Howes et al. 2011 |
| Centerville River System | Howes et al. 2006c |
| Falmouth Inner Harbor Embayment System | Howes et al. 2013a |
| Fiddlers Cove and Rands Harbor Embayment Systems | Howes et al. 2013b |
| Great/Perch Pond, Green Pond, and Bournes Pond | Howes et al. 2005a |
| Herring River Embayment System | Howes et al. 2013c |
| Lewis Bay System | Howes et al. 2008a |
| Little Pond System | Howes et al. 2006d |
| Namskaket Marsh Estuarine System | Howes et al. 2008b |
| Nauset Harbor Embayment System | Howes et al. 2012a |
| Oyster Pond System | Howes et al. 2006e |
| Parkers River Embayment System | Howes et al. 2010b |
| Phinneys Harbor – Eel Pond – Back River System | Howes et al. 2006f |
| Pleasant Bay System | Howes et al. 2006a |
| Popponesset Bay | Howes et al. 2004 |
| Quissett Harbor Embayment System | Howes et al. 2013d |
| Rock Harbor Embayment System | Howes et al. 2008c |
| Salt Pond Embayment System | Howes et al. 2014a |
| Sandwich Harbor Estuary | Howes et al. 2015a |
| Stage Harbor, Sulphur Springs, Taylors Pond, Bassing Harbor, and Muddy Creek | Howes et al. 2003, Howes et al. 2007 |
| Swan Pond River Embayment System | Howes et al. 2012b |
| Three Bays | Howes et al. 2005b |
| Waquoit Bay and Eel Pond Embayment System | Howes et al. 2013e |
| Wellfleet Harbor Embayment System | Howes et al. 2017a |
| West Falmouth Harbor | Howes et al. 2006b |
| Wild Harbor Embayment System | Howes et al. 2013f |
| **Region: South Coast and Buzzards Bay** | |
| Nasketucket Bay Embayment System | Howes et al. 2013g |
| New Bedford Inner Harbor Embayment System | Howes et al. 2015b |
| Slocum and Little River Estuaries | Howes et al. 2012c |
| Wareham River, Broad Marsh and Mark's Cove Embayment System | Howes et al. 2014b |
| Westport River Embayment System | Howes et al. 2013h |

A literature search that employed online commercial databases, literature search tools, and internet search tools was conducted to obtain peer-reviewed and other published literature (e.g. government and industry technical reports and manuals, books, book chapters) on methods for studying, characterizing, and monitoring benthic habitat. Key search terms and phrases were used to conduct methodical queries of databases and the internet. Examples of terms and phrases used in the search include: "marine benthic sampling methods", "marine benthic methods", "marine infaunal sampling methods", "study marine benthos", "marine infaunal techniques", "marine macrofauna techniques ", "marine meiofauna techniques", "marine benthic indicators", "marine benthic index", "B-IBI", "VPBI", and "AMBI". Reference listings from relevant documents were also used to identify important earlier work on the same topics.

A Technical Advisory Committee (TAC) was convened and contributed expertise and input on technical aspects of sampling methodology and approach development. The TAC was comprised of representatives from Massachusetts Office of Coastal Zone Management, Massachusetts Executive Office of Energy and Environmental Affairs, the Buzzards Bay Coalition, and Massachusetts Maritime Academy. Sampling methodology and approaches were refined based on comments received from the TAC.

# 3 Findings

The literature review of the MEP guidance documents and technical reports, other benthic monitoring programs, and published sources provided a wealth of information on the methods available for updating the MEP benthic infaunal monitoring and development of a revised approach. Summarized below are the current MEP methods and program descriptions of the other benthic monitoring programs reviewed. Documentation for some of the larger programs is extensive; in these instances relevant documents were referenced so that project material (e.g. standard operating procedures and quality assurance plans) could be examined. The review of the published literature focused on benthic sample collection methods, sieve mesh size, preservatives, and types of benthic habitat indicators and indices currently available.

## 3.1 MEP Documents

A QAPP was developed in 2003 to define the tasks required to fulfill all of 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. The model when properly parameterized, calibrated and validated for a given embayment becomes a nitrogen management planning tool which supports TMDL analysis, suggests "solutions" for the protection or restoration of nutrient related water quality, and allows testing of management scenarios (Howes et al. 2001). Technical reports for each of the assessed embayments convey the embayment specific results generated from the implementation of the Linked Watershed-Embayment Approach. The assessment of embayment health and the determination of nutrient thresholds capable of maintaining or restoring the ecological health for a specific embayment were based on habitat indicators. The primary habitat indicators identified by Howes et al. (2003) for the purposes of the MEP and the use of the Linked Watershed-Embayment Management Model Approach to evaluate embayment health and nitrogen assimilative capacity were:

- plant presence and diversity (eelgrass, macroalgae, etc.)
- animal species presence and diversity (finfish, shellfish, infauna)
- nutrient concentrations (nitrogen species)
- chlorophyll concentration, and
- dissolved oxygen levels in the embayment water column.

These indicators were then combined with a full water quality synthesis and projections of future conditions based upon water quality modeling to develop site-specific thresholds for assessed embayments (Howes et al. 2003).

### *3.1.1 MEP Quality Assurance Project Plan*

The MEP QAPP presents the methods used to assess benthic habitats in the southeastern Massachusetts embayments since 2003. The indicator program consists of 3 components: benthic animal surveys, eelgrass and macroalgal surveys, and continuous dissolved oxygen recordings (30 days) from key sites. The goal was to provide data needed to conduct habitat assessments throughout the sub-embayments of each embayment system and to determine long-term changes. The results from each embayment's indicator sampling were to be directly comparable to the others within the Estuaries Project and other studies conducted by the EPA and NOAA (Howes and Samimy 2003). The MEP QAPP and its revisions also contain embayment-specific sampling and analysis plans for Embayments 1 through 64 (Howes and Samimy 2003, UMass Dartmouth 2004, Howes and Samimy 2005). The embayment-specific plans contain a brief system description, data collection targets, MEP analysis and goals, and maps of the planned sampling locations (Howes and Samimy 2003, UMass Dartmouth 2004, Howes and Samimy 2005).

The objectives for the habitat indicator measurements were primarily to identify and count numbers of benthic infauna organisms or map macrophyte distribution/s (Howes and Samimy 2003). The method and minimum performance criteria for inclusion for each of the relevant parameters into the habitat assessment program database were presented in Table B.6-1 of the MEP QAPP (Howes and Samimy 2003) and amended in the Year 2 QAPP (UMass Dartmouth 2004, Howes and Samimy 2005). Figure 1 presents the method and minimum performance criteria for the current MEP Habitat Assessment Program.

**Figure 1. Habitat Assessment Program, field parameters measured and data objectives (Table B.6-1 from Howes and Samimy 2005).**

| Parameter | Method | Detection Limits | Frequency | QC Samples | Acceptable %Recovery |
|---|---|---|---|---|---|
| Macrophytes | Video Survey | Individual shoots | 1 survey May-Oct. | Spot checks by diver | 95% |
| Benthic Animals | Van Veen Grab or Coring as appropriate Microscope sorting | 1 animal | 1 survey Aug-Oct | Spot checks by different specialist | 95% |
| Dissolved Oxygen | Rapid Pulse Clark Electrode | 0.2 mg/L | 15 min. for 1-2 months, July-Sept. | Winkler Titration of water from sensor depth(start,mid,end of mooring deployment) | 75 % |
| Temperature | Bead Thermistor (sensitivity of 0.1°C) | NA | 15 min. for 1-2 months, July-Sept. | Calibrated to certified thermometer prior to deployment | 75 % |
| Conductivity/ Salinity | 2-electrode platinum conductivity cell 1 cm. Path length | 0.1 mS/cm | 15 min. for 1-2 months, July-Sept. | Whole water collected at sensor depth (start,mid,end of mooring deployment) | 75 % |
| Depth | Differential Strain gauge transducer | 0.02 m | 15 min. for 1-2 months, July-Sept. | Calibrated to measured tape level at beginning and end of deployment | 75 % |
| Chlorophyll a | Fixed wavelength fluorometer, 470 nm | 0.1 ug/L | 15 min. for 1-2 months, July-Sept. | Chlorophyll extraction (start,mid,end of mooring deployment) | 75 % |

Below are summaries of the parameter methods for the three benthic habitat indicators presented in the MEP QAPP (Howes and Samimy 2003).

## Benthic Animal Surveys

Benthic animal communities can be used to assess the level of habitat health from healthy (low organic matter, high D.O.) to highly stressed (high organic matter, low D.O.). The basic concept is that certain species or species assemblages reflect the quality of their habitat (Howes et al. 2006a and b).

Field Methods – Benthic infauna sampling sites were paired to the sediment regeneration sites (Howes and Samimy 2003). At each sampling site within an embayment, triplicate samples were collected once either by Van Veen grab (25 cm x 25 cm) or diver collected cores (15 cm id) from August through October. Samples were washed with seawater through a 0.300 mm sieve to separate the animals and then washed with seawater into 1 gallon polyethylene wide mouth jars, dyed with a vital stain, and preserved with buffered ethanol. Field data were recorded in field books or on standardized data sheets on-site at the time the measurements were taken.

Analysis – The jars were then returned to the laboratory where duplicate samples were hand sorted. After a sample was sorted, a different technical member checked the sample for "missed" organisms. The harvested organisms were then identified based upon standard keys (e.g. Smith 1964, Gosner 1971) and local museum collections (e.g. Marine Biological Laboratory Museum Collection and the Woods Hole Oceanographic Benthic Collection). If the duplicate samples differed by more than 30%, the third replicate sample was sorted. Species identification was periodically checked by an independent expert on 5% of the samples. All species of uncertain identification were confirmed by an independent expert. Cross checks were also made using the museum collections. Historic data was used for comparison to present data to determine trends. If the historic data was collected using different methods, then qualitative statements were made. The benthic animal data were held in spreadsheet format for each embayment on CD-ROM in the SMAST Project Library and with the Technical Coordinator.

#### Macrophyte Survey

Field Methods - The MEP QAPP (Howes and Samimy 2003) contains a detailed Eelgrass Surveying Protocol in B-1 Section L. These methods are a part of the MassDEP Wetlands Conservancy Program that conducts the statewide seagrass mapping effort, for more information on this program see Costello and Kenworthy (2011). MassDEP has committed to continuing the eelgrass monitoring in coastal estuaries in 3-year periods as part of the Wetlands Conservancy Program. Therefore, no further discussion of this habitat indicator will be included in this report as any revisions to the methodology used for eelgrass monitoring will be implemented through the Wetlands Conservancy Program.

#### Continuous Dissolved Oxygen Recordings

Field Methods - The MEP deploys ENDECO/YSI 6600 sensor systems in the target embayments from July 1 to September 15 of the field data collection year/s to measure dissolved oxygen, temperature, salinity and chlorophyll a. Sensors are located within "areas of concern" and other areas throughout each system, as indicated from the long-term monitoring baseline. The sites are selected based upon measured dissolved oxygen levels of <4mg/L by water quality monitoring programs or indications that a low dissolved oxygen environment may exist. See the MEP QAPP (Howes and Samimy 2003) for more information on this project component. Many communities have committed to on-going water quality monitoring programs, as result this habitat indicator will only be discussed in this report in instances where dissolved oxygen data collection is directly connected with benthic infaunal surveys.

### 3.1.2 MEP Technical Reports

The MEP technical reports (Table 1) present the embayment specific results generated from the implementation of the MEP Linked Watershed-Embayment Approach. As part of this approach, a habitat assessment was conducted on each of the embayments based upon available water quality monitoring data, historical changes in eelgrass distribution, time-series water column oxygen measurements, and the benthic community structure. The primary results in these reports were: 1) a current quantitative assessment of embayment health, 2) identification of all nitrogen sources to embayment waters, 3) nitrogen threshold levels for maintaining Massachusetts Water Quality Standards within embayment waters, 4) analysis of watershed nitrogen loading reduction to achieve the N threshold concentrations in embayment waters, and 5) a functional calibrated and validated Linked Watershed-Embayment modeling tool that can be used for evaluation of nitrogen management alternatives (to be developed by the Town) for the restoration of the embayment system (Howes et al. 2006a and b).

Our review of MEP technical reports presented in Table 1 focused on the benthic infauna analysis that was used in part to determine embayment ecological health. The assessment of embayment ecological health is presented in Section VII of these technical reports and contains an overview of the concept of benthic animal indicators, the number of benthic infaunal sampling locations collected in the embayment, a map showing the benthic infaunal sampling locations, and the benthic infauna analysis results. The benthic infauna analyzes were based on species life-history information and animal-sediment relationships (Rhoads and Germano 1986) and a variety of field studies within southeastern Massachusetts waters including Sanders (1960), Sanders et al. (1980), Tian et al. (2009), Howes and Taylor (1990), and Howes et al. (1997; Howes et al. 2006a and b, Howes et al. 2013d). Distribution of species (types of species present) and the population density (total number of individuals) were coupled with the level of diversity (H’) and evenness (E) of the benthic community to determine the infaunal habitat quality of each embayment. Habitat quality for each embayment was classified as representative of healthy, transitional, or stressed conditions (Howes et al. 2006a and b, Howes et al. 2013d). Four levels of habitat condition were determined: healthy habitat conditions/high quality habitat conditions, moderately impaired, significantly impaired, and severely degraded (Howes et al. 2006a and b, Howes et al. 2013d).

The results presented in the Section VII benthic infauna analysis provide broad embayment and/or sub-embayments characterization of the benthic infaunal community. The characterizations include discussions on the level of habitat condition, types of dominant taxa present, and the diversity and evenness values calculated for the sampling stations and a section on the shellfish resource areas for some embayments (Howes et al. 2013d). The habitat conditions determined by each habitat health indicator for each overall embayment and its sub-embayments are summarized in Section VIII, Table VIII-1 of these reports. Lastly, embayment specific primary and secondary sentinel locations are identified in Section VIII so that restoration of this one site in an embayment would necessarily bring the other regions of the system to acceptable habitat quality levels (Howes et al. 2006a and b, Howes et al. 2013d).

**Summary -** The following methods and data can be used as the program moves forward into post- TMDLs implementation assessment: 1) embayment specific benthic infaunal sampling stations, 2) embayment specific primary and secondary sentinel locations, 3) use a Van Veen grab for benthic infaunal sample collection, 4) collection time (August – October), 5) general organism separation, preservation, and identification methods, 6) the use of the types of species present, the total number of individuals, diversity (H’) and evenness (E) to help determine the infaunal habitat quality, and 7) the reported habitat conditions determined by the habitat health indicators for each assessed embayment presented in Tables VIII-1.

## 3.2 U.S. Environmental Protection Agency National Coastal Condition Assessment

The National Coastal Condition Assessment (NCCA) provides a comprehensive assessment for coastal waters of the United States. It was designed to: 1) assess the condition of the Nation's coastal and estuarine waters at national and regional scales; 2) identify the relative importance of selected stressors to coastal and estuarine water quality; 3) evaluate changes in condition from previous National Coastal Assessments starting in 2000; and 4) help build State and Tribal capacity for monitoring and assessment and promote collaboration across jurisdictional boundaries (US EPA 2015a). The NCCA uses 12 indicators (e.g. hydrographic profile, light attenuation, chlorophyll-a, dissolved nutrients, sediment assessment, and benthic macroinvertebrate assemblage) in the marine and Great Lakes coastal surveys. Measurements and samples are collected at preselected sampling sites during the index period of June through the end of September (US EPA 2015a). Complete documentation of overall project management, design, methods, and standards is contained in the following four documents[1]:

- NCCA 2015: Quality Assurance Project Plan (EPA-841-R-14-005; US EPA 2016a)
- NCCA 2015: Site Evaluation Guidelines (EPA-841-R-14-006; US EPA 2015b)
- NCCA 2015: Field Operations Manual (EPA-841-R-14-007; US EPA 2015a)
- NCCA 2015: Laboratory Operations Manual (EPA-841-R-14-008; US EPA 2016b)

Our review of these documents focused on the sediment assessment (grain size, total organic carbon [TOC], organics and metals), benthic macroinvertebrate assemblage (composition and abundance) indicators, and general project management and design. Program sampling locations were selected using an unequal probability stratified design (Generalized Random Tessellation Stratified) see Olsen (2010), and US EPA (2015a and b). The NCCA 2015 Field Operations Manual Section 12 (Sediment Collection) contains the detailed equipment list, sampling procedure, and processing procedure for sample collection of the sediment assessment and benthic macroinvertebrate assemblage indicators. Sediment samples for these two indicators were collected using a 0.04 $m^2$ stainless steel Young-modified Van Veen grab (or similar) sampler. A minimum of 3 liters (L) of marine sediment was collected for the sediment assessment analyses (US EPA 2015a). The NCCA 2015 Laboratory Operations Manual Section 4.0 (Benthic Macroinvertebrates) contains the detailed procedures for benthic macroinvertebrate sample processing which include health and safety, taxonomic identification, data entry, and quality measures (US EPA 2016b). The NCCA 2015 Laboratory Operations Manual Section 6.0 (Sediment Contaminant, Grain Size, and TOC Analyses) contains the analysis requirements for sediment samples (contaminants, grain size, and TOC) which include health and safety warnings, laboratory analysis requirements, data entry, and quality measures (US EPA 2016b). Degraded benthic habitats were distinguished from undegraded habitats using the regional benthic indices Engle et al. (1994), Weisberg et al. (1997), Engle and Summers (1999), Van Dolah

[1] Available at https://www.epa.gov/national-aquatic-resource-surveys/manuals-used-national-aquatic-resource-surveys#National Coastal Condition Assessment.

et al. (1999), and Hale and Heltshe (2008) developed by the Environmental Monitoring and Assessment Program (EMAP) National Coastal Assessment (NCA) the precursor to the NCCA(US EPA 2016a).

**Summary** - Site selection criteria, field collection methods, laboratory methods, and the northeast benthic indices developed for the NCCA program could be useful in updating the MEP benthic infauna survey procedures and developing a streamlined or tiered approach. The continued use of a modified Van Veen grab in August and September would be appropriate for an updated MEP benthic sampling procedure.

## 3.3 National Oceanic and Atmospheric Administration National Status and Trends Program

The National Status and Trends (NS&T) Program was designed to describe the current status of, and detect changes in, the environmental quality of the nation's estuarine and coastal waters through environmental monitoring, assessment and related research. This program included three nationwide projects: (1) Benthic Surveillance, (2) Mussel Watch, and (3) Bioeffects. The Benthic Surveillance Project was discontinued in 1993; the other two programs continue.

### *3.3.1 Benthic Surveillance Project*

The Benthic Surveillance Project (1984 to 1992) defined the geographic distribution of contaminant concentrations in sediments and the tissues of marine organisms (e.g. fish), and documented biological responses to contamination. Surficial sediment samples were collected annually from 1984 through 1986 from sites in nearshore United States waters to monitor contaminant levels. Starting in 1987, sites were monitored every other year. Collections located in the Northeast (Chesapeake Bay through Maine) occurred between April and June using a specially constructed box corer, a standard Smith- MacIntyre grab, or a specially constructed Kynar-coated Young-modified Van Veen grab (Lauenstein and Young 1986, Lauenstein and Cantillo 1993, Lauenstein et al. 1996). Sediment samples were analyzed for major and trace elements, organic contaminants, total organic carbon, moisture content, and particle size distribution (Lauenstein and Cantillo 1993). Documentation of the overall project, sampling procedures, quality assurance program, and specimen bank are contained in the following documents:

- National Status and Trends Program for Environmental Quality Benthic Surveillance Project: Cycle III Field Manual. NOAA Technical Memorandum NOS OMA 28 (Lauenstein and Young 1986)
- Sampling and Analytical Methods of the National Status and Trends Program National Benthic Surveillance and Mussel Watch Projects 1984-1992. Volume I Overview and Summary of Methods. NOAA Technical Memorandum NOS ORCA 71 (Lauenstein and Cantillo 1993)
- National Status and Trends Program Specimen Bank: Sampling Protocols, Analytical Methods, Results, and Archive Samples. NOAA Technical Memorandum NOS ORCA 98 (Lauenstein et al. 1996)

### *3.3.2 Mussel Watch Project*

The Mussel Watch Project (MWP; 1986 to present) monitors the coastal waters for chemical contaminants and biological indicators of water quality through the collection and analysis of indigenous bivalve mollusks (oysters and mussels) and sediment. Currently, there are approximately 300 core sites along the coast. Initially, sites were established in areas intended to represent general conditions of broad coastal areas, therefore not located near specific pollution outfalls. In recent years however, the MWP has expanded to meet a broader mission and has included sites in areas known to be directly impacted by outfalls and/or known pollution sources (Apeti et al. 2012). The MWP collects fine grained surface sediment (> 20% fine material) from non-exposed sub-tidal sites. Sediments are collected using a cleaned (with acetone) Van Veen grab, PONAR-grab or hand held box-core. The grab sediments are inspected for disturbance and then a stainless steel scoop is used to remove the top 2 to 3 cm sediment materials. Samples are analyzed for organic contaminants and trace metals along with the secondary measurements of grain size and TOC (Apeti et al. 2012). Compounds have been added over time, approximately 140 compounds are now monitored including polybrominated diphenyl ethers (PBDEs) that are being studied using the historic specimen bank samples and current samples to determine their spatial distribution (Kimbrough et al 2008). Recent sampling procedures are contained in the following document[2]:

- National Status and Trends Mussel Watch Program: Sampling Methods 2012 Update. NOAA Technical Memorandum 134 (Apeti et al. 2012).

**Summary** – Surficial sediment sample collection methods used in the Benthic Surveillance and Mussel Watch projects could be applicable in updating the MEP benthic infauna survey procedures. An inspection for disturbance of grab sediments is a procedure that should be considered for inclusion in an updated MEP benthic method. In addition, the site selection criteria used in the broadened Mussel Watch Project could be useful in selecting re-assessment locations for the MEP embayments.

## 3.4 Massachusetts Office of Coastal Zone Management Seafloor and Habitat Mapping Program

The Massachusetts Office of Coastal Zone Management (CZM) Seafloor and Habitat Mapping Program has collected sediment and infaunal benthic samples since 2010 to categorize and map marine resources and habitats in Commonwealth waters. The information is used to protect these resources and ensure that development projects in Massachusetts ocean waters avoid and minimize potential impacts. High-resolution bathymetry and surficial geology with sediment and organism data were used to map the seafloor habitats. Sediment and infaunal benthic samples were used to validate the sediment maps. The CZM and Division of Marine Fisheries (DMF) conducted seafloor sediment surveys for the following target areas: June 2010 - Massachusetts Bay and Cape Cod Bay; September 2011 - southern Cape Cod Bay, south of the

[2] Available at https://coastalscience.noaa.gov/data_reports/national-status-trends-mussel-watch-program-sampling-methods-2012-update/.

Islands including Vineyard Sound, and Buzzards Bay; and August 2012 - between Boston Harbor and the New Hampshire border (Normandeau 2010, AECOM 2012, 2013[3]). In 2010, a 0.04 $m^2$ Ted Young-modified Van Veen grab was used to collect two grabs at each station; one grab was collected for grain size analysis and the other for infaunal analysis (Normandeau 2010). Since 2011 the Program has used a 0.1-$m^2$ modified Van Veen grab attached to the USGS SEABed Observation and Sampling System (SEABOSS). The resulting sample was divided using a sheet of plexiglass; one side (volume approximately 0.06 $m^2$) was used for grain size analysis and the other side (approximately 0.04 $m^2$) was used for infaunal analysis (AECOM 2012, 2013). SEABOSS consists of forward- and down-looking video cameras, a digital still camera, and a modified Van Veen grab contained within a stainless steel framework, measuring 110 × 110 centimeters (cm) and 118 cm tall and weighing 167 kilograms (368 pounds) overall (Ackerman et al. 2015).

Basic descriptive statistics were calculated for each infaunal sample. PRIMER software was used to calculate abundance of each taxon, number of taxa, the diversity index Shannon's H′ ($\log_e$), and Pielou's evenness value J′. Similarity analyses using the Bray-Curtis algorithm based on 4th-root transformed data were performed for each study area. The SIMPROF routine was used to identify faunal assemblages by detecting internally consistent groups that were significantly different from other groups of stations. SIMPER analysis was performed in order to identify the contribution of individual taxa to the overall dissimilarity among groups. Non-metric multi-dimensional scaling (MDS) diagrams were generated to depict the relationship of stations in two-dimensional space in terms of the major cluster groups and environmental factors of interest (Normandeau 2010, AECOM 2012, 2013).

Benthic images were classified according to a modified version of the Coastal and Marine Ecological Classification Standard (CMECS) Version 4.0, Benthic Biotic Component. CMECS is a hierarchal system that provides a means for classifying ecological units using a standard format. Biotic classification is defined by the dominance of stable, fixed (sessile), or slow moving species visible in the photo. Two co-occurring biotic groups and two associated taxa are also included in the classification system (FGDC 2012, Hubbard 2016, Commonwealth of Massachusetts 2018).

**Summary** – The continued use of a modified Van Veen grab in August and September for sediment and benthic infaunal collection would be appropriate in an updated MEP benthic sampling procedure. The use of PRIMER or a similar statistical software package for infaunal community analysis should be considered for inclusion in an updated benthic survey approach. Lastly, benthic image collection using a high definition (HD) digital camera with analysis following the CMECS Version 4.0, Benthic Biotic Component or a modified version should be considered for inclusion during the development of a streamlined or tiered approach.

[3] Available at https://www.mass.gov/service-details/czm-seafloor-and-habitat-mapping-publications.

## 3.5 Massachusetts Water Resources Authority Harbor and Outfall Monitoring Program

The Massachusetts Water Resources Authority (MWRA) is a Massachusetts public authority established in 1984 by an act of the Massachusetts Legislature to provide wholesale water and sewer services to metropolitan Boston communities. MWRA is required to conduct environmental monitoring in Boston Harbor, Massachusetts Bay, and Cape Cod Bay to control water pollution from of the Deer Island Wastewater outfall discharge and ensure compliance with the federal Clean Water Act. MWRA has been monitoring Boston Harbor and its tributaries since 1989 (MWRA 2017).

An initial monitoring plan was developed for baseline monitoring which has been modified over time to cover post-diversion (outfall) monitoring (MWRA 2010, Nestler et al. 2014). The MWRA Ambient Monitoring Program consists of four components: effluent monitoring, water column monitoring, benthic monitoring, and fish and shellfish monitoring (MWRA 2010). In Boston Harbor, the focus is on tracking the potential recovery of the benthic communities after pollution abatement (Nestler et al. 2014, Rutecki et al. 2017). Normandeau's review of the MWRA Ambient Monitoring Program focused on the benthic monitoring component.

Documentation of sediment chemistry analysis and benthic monitoring project management, design, and methods is contained in the following documents[4]:

- Quality Assurance Project Plan – Sediment Chemistry Analyses for Harbor and Outfall Monitoring (MWRA 2014-02; Constantino et al. 2014)
- Quality Assurance Project Plan – Benthic Monitoring 2014–2017 (MWRA 2014-03; Nestler et al. 2014)
- Boston Harbor Benthic Monitoring Report: 2016 Results (MWRA 2017-10; Pembroke et al. 2016)
- Quality Assurance Project Plan – Benthic Monitoring 2017–2020 (MWRA 2017-06; Rutecki et al. 2017).

The sediment chemistry analysis samples are collected in August from stations within Boston Harbor and Massachusetts Bays in the nearfield and farfield of the outfall using a Kynar coated Ted Young-modified Van Veen grab. The upper 0-2 cm is subsampled with a Kynar coated scoop. At the start and every third year subsequently, samples are analyzed for total organic carbon (TOC), grain size, *Clostridium perfringens* and chemical contaminants. Samples in other years are analyzed only for TOC, grain size, and *C. perfringens* (Constantino et al. 2014). A 0.1-$m^2$ grab is used to collect all soft-bottom sediment samples for chemical analyses (organic and inorganic). A 0.04-$m^2$ grab may be used to collect samples for TOC, grain size, and *C. perfringens*, as long as sufficient sample volume can be obtained (Nestler et al. 2014, Rutecki et al. 2017). The MWRA QAPP – Sediment Chemistry Analyses for Harbor and Outfall

[4] Available at http://www.mwra.state.ma.us/harbor/enquad/trlist.html.

Monitoring (MWRA 2014-02) contains sample handling and custody requirements, analytical methods, data management, and oversight requirements (Constantino et al. 2014).

The benthic monitoring program includes traditional bottom grab surveys in Boston Harbor and Outfall nearfield and farfield soft-bottom habitats for physical, chemical, and biological data. Boston Harbor and Outfall nearfield sediment profile images (SPI); and an Outfall nearfield benthic remotely operated vehicle (ROV) survey provide semi-quantitative hard-bottom community data (Nestler et al. 2014, Rutecki et al. 2017). Methods and quality assurance are routinely scrutinized by the MWRA managers, ensuring a high level of consistency. Grab sampling is conducted in August using a 0.04-$m^2$ Ted Young-modified Van Veen grab sampler to collect soft-bottom sediment samples for infaunal analysis. SPI are collected in August using a digital sediment profile camera to evaluate sediment grain size, sediment layering, surface and subsurface fauna and structures, approximate prism penetration, approximate surface relief, approximate aRPD, and other major discernable patterns. Hard-bottom community images are collected in June using a ROV equipped with high definition video camera (Nestler et al. 2014, Rutecki et al. 2017). Field processing and storage of samples for the MWRA Boston Harbor Benthic Surveys and Outfall Benthic Surveys are summarized in Figures 2 and 3. The MWRA QAPP – Benthic Monitoring 2014–2017 and 2017–2020 (MWRA 2014-03 and MWRA 2017-06, respectively) contain sampling methods, sample handling and custody requirements, analytical methods, data management, and oversight requirements (Nestler et al. 2014, Rutecki et al. 2017). Benthic infaunal data are analyzed using Shannon-Weiner (H') biodiversity index, classification (cluster analysis) by hierarchical agglomerative clustering with group average linking, and ordination by MDS and Bray-Curtis similarity. Benthic habitat conditions in Boston Harbor from SPI are measured by the Organism Sediment Index (OSI) following Rhoads and Germano (1986; Pembroke et al. 2016).

**Summary** – The MWRA Ambient Monitoring Program suggests that the benthic infaunal sampling methods, laboratory and analysis methods, and program organization may provide useful templates for updating the MEP benthic infaunal survey procedures. The MWRA benthic infaunal sampling methods indicate that the continued use of a modified Van Veen grab would be appropriate for an updated MEP benthic sampling procedure. SPI and HD digital images should be considered for inclusion during the development of a streamlined or tiered approach.

**Figure 2. Processing and storage of field samples taken on MWRA Boston Harbor Benthic Surveys collection years 2017-2019 (taken from Rutecki et al. 2017, Table 9).**

| Activity | Task 5.1<br>Harbor Infaunal Survey | Task 5.2<br>Harbor Reconnaissance Survey (SPI) |
|---|---|---|
| Stations | 9 (T01–T08 and C019, see Table 2) | 61 ( T01–T08, C019, R02–R53, see Table 2) |
| Station location and time | Record beginning and ending location and time of station visit, and location of individual samples | 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 | As for Task 5.1 |
| Marine mammals | Note incidental observations | As for Task 5.1 |
| Sampling: Gear | 0.04-$m^2$ Ted Young-modified van Veen grab sampler | Sediment profile camera |
| Sampling: Measurements | Record penetration depth to nearest 0.5 cm and sediment volume to nearest 0.5 L | Record prism penetration (1 cm) |
| Sampling: Sediment texture | Describe qualitatively | Estimate from images (see Section B4.2) |
| Sampling: aRPD depth | Record visual estimate of aRPD to nearest 0.5 cm | Visual estimate |
| Faunal Samples: Number | 3 at each station | 3 images at each station |
| Faunal Samples: Processing | Rinse over 300-µm-mesh sieve; fix in 10% buffered formalin | Check memory card for images |
| Faunal Samples: Storage | Clean, labeled plastic jars; ambient temperature | NA |
| Chemistry (Ancillary) /Microbiology Samples (All): Number | 1 at each station | NA |
| Chemistry Samples (Ancillary): Processing | Use Kynar-coated scoop to collect upper 0–2 cm from grab, homogenize and collect ~50 mL subsample for TOC and ~500 mL for grain size | NA |
| Chemistry Samples (Ancillary): Storage[1] | Clean, labeled glass jar. Freeze TOC at -20°C; refrigerate grain size. Holding time is 28 days for both TOC and grain size. | NA |
| Microbiology Samples: Processing | Use Kynar-coated scoop to collect upper 0–2 cm from grab, homogenize and collect ~25 mL subsample | NA |
| Microbiology Samples: Storage[1] | Sterile sample bottle; refrigerate at 4°C.[2] Holding time not defined. | NA |

[1]Sediment samples will be delivered to MWRA's Department of Laboratory Services (DLS) for testing. The analysis of certain parameters may be performed by contracted laboratories as detailed in Constantino et al. (2014), and updates as issued by DLS.

[2]*C. perfringens* may be stored frozen, but then must not be thawed until analyses are performed.

**Figure 3. Field processing and storage of samples taken on MWRA Outfall Benthic Surveys for collection years 2017-2019 (taken from Rutecki et al. 2017, Table 10).**

| Activity | Nearfield Benthic Survey (Task 6.1) | Farfield Benthic Survey (Task 6.1) | Nearfield SPI Survey (Task 6.2) | Nearfield Hard-bottom Survey (Task 6.3) |
|---|---|---|---|---|
| Stations | 11 (Table 3) | 3 (Table 3) | 23 (Table 3) | 18 waypoints on 6 transects ( T1, T2, T4, T6, T7, T8) plus 5 single waypoints: T9, T10, T11, T12, diffuser #44 (Table 4) |
| Station location and time | Record beginning and ending location and time of station visit and location of individual samples | Record beginning and ending location and 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 |
| 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 |
| Marine mammals | Note incidental observations | Note incidental observations | Note incidental observations | Note incidental observations |
| Sampling: Gear | Ted Young-modified van Veen grab sampler | Ted Young-modified van Veen grab sampler | Digital sediment profile camera | ROV equipped with video camera |
| Sampling: Measurements | Record penetration to nearest 0.5 cm and sediment volume to nearest 0.5 L | Record penetration to nearest 0.5 cm and sediment volume to nearest 0.5 L | Record prism penetration | Record ROV position, depth, heading |
| Sampling: Sediment texture | Describe qualitatively | Describe qualitatively | Estimate from images (see Section B2.2.3) | Not Applicable (NA) |
| Sampling: apparent RPD depth | Record visual estimate (0.5 cm) | Record visual estimate (0.5 cm) | Estimate from images (see Section B2.2.3) | NA |
| Faunal Samples/Images: Number | 2 at each station | 2 at each station | 3 at each station | 20 min video analog and digital per waypoint |
| Faunal Samples/Images: Processing | Rinse over 300-µm-mesh sieve; fix in 10% buffered formalin | Rinse over 300-µm-mesh sieve; fix in 10% buffered formalin | Preview images within 3 business days of survey completion (see section B2.2.3) | Analog video saved to DVD<br>Digital video save to external hard drive. |
| Faunal Samples/Images: Storage | Clean, labeled plastic jar; ambient temperature | Clean, labeled plastic jar; ambient temperature | CD | DVD |
| Chemistry/ microbiology Samples: Number | 1 at each station | 1 at each station | NA | NA |

**Figure 3. Continued (taken from Rutecki et al. 2017, Table 10).**

| Activity | Nearfield Benthic Survey (Task 6.1) | Farfield Benthic Survey (Task 6.1) | Nearfield SPI Survey (Task 6.2) | Nearfield Hard-bottom Survey (Task 6.3) |
|---|---|---|---|---|
| Chemistry Samples (Organics): Processing | Use Kynar-coated scoop to collect upper 0–2 cm from grab, homogenize and collect ~125 mL subsample | Use Kynar-coated scoop to collect upper 0–2 cm from grab, homogenize and collect ~125 mL subsample | NA | NA |
| Chemistry Samples (Organics): Storage[1] | Clean labeled 250 ml (8 oz) glass jar with Teflon-lined screw cap; freeze (-20° C); holding time is 1 year to extract (if samples are frozen) and 40 days from extraction to analysis | Clean labeled 250 ml (8 oz) glass jar with Teflon-lined screw cap; freeze (-20° C); holding time is 1 year to extract (if samples are frozen) and 40 days from extraction to analysis | NA | NA |
| Chemistry Samples (Metals): Processing | Use Kynar-coated scoop to collect upper 0–2 cm from grab, homogenize and collect ~100 mL subsample | Use Kynar-coated scoop to collect upper 0–2 cm from grab, homogenize and collect ~100 mL subsample | NA | NA |
| Chemistry Samples (Metals): Storage [1] | Clean, 125 ml (4 oz. plastic labeled I-Chem© jar; freeze (-20° C); holding time is 6 months to preparation; Hg holding time is 28 days to preparation. | Clean, 125 ml (4 oz. plastic labeled I-Chem© jar; freeze (-20° C); holding time is 6 months to preparation; Hg holding time is 28 days to preparation. | NA | NA |
| Chemistry Samples (Ancillary): Processing | Use Kynar-coated scoop to collect upper 0–2 cm from grab, homogenize, and collect ~50 mL subsample for TOC and ~500 mL for grain size. | Use Kynar-coated scoop to collect upper 0–2 cm from grab, homogenize, and collect ~50 mL subsample for TOC and ~500 mL for grain size. | NA | NA |
| Chemistry Samples (Ancillary): Storage[1] | Clean, labeled, wide-mouth glass jar (125 ml (4 oz) for TOC and 500 ml (16 oz) for grain size); freeze TOC, refrigerate grain size.<br>Holding time is 28 days for both TOC and grain size | Clean, labeled, wide-mouth glass jar (125 ml (4 oz) for TOC and 500 ml (16 oz) for grain size); freeze TOC, refrigerate grain size.<br>Holding time is 28 days for both TOC and grain size | NA | NA |
| Microbiology Samples: Processing | Use Kynar-coated scoop to collect upper 0–2 cm from grab, homogenize and collect ~25 mL subsample | Use Kynar-coated scoop to collect upper 0–2 cm from grab, homogenize and collect ~25 mL subsample | NA | NA |
| Microbiology Samples: Storage[1] | Sterile sample bottle; refrigerate at 4°C[2], holding time not defined. | Sterile sample bottle; refrigerate at 4°C[2], holding time not defined. | NA | NA |

[1]Sediment samples will be delivered to MWRA's Department of Laboratory Services (DLS) for testing. The analysis of certain parameters may be performed by contracted laboratories as detailed in Constantino et al. (2014), and updates as issued by DLS.

[2]*C. perfringens* may be stored frozen, but then must not be thawed until analyses are performed.

## 3.6 Southern California Coastal Water Research Project

The Southern California Coastal Water Research Project (SCCWRP) is a research institute founded as a public agency in 1969 to develop a scientific foundation for informed water-quality management in Southern California and beyond. SCCWRP organizes and participates in several collaborative regional monitoring programs including the Southern California Bight Regional Monitoring Program.

### *3.6.1 The Southern California Bight Regional Monitoring Program*

Southern California Bight Regional Monitoring Program is an ongoing cooperative program, involving nearly 100 agencies including international and volunteer organizations, that occurs on a five year basis. The most recent surveys were completed in 2013 and 2018 (Bight '13 and Bight '18). At the time of review, only documents from the 2013 survey were publically available. The Bight '13 Survey was organized into five technical components: 1) Contaminant Impact Assessment; 2) Shoreline microbiology; 3) Water quality; 4) Debris, and 5) Rocky reefs (Gillett et al. 2017). The review of this program focused only on the Contaminant Impact Assessment that concentrated on sediment contaminants and associated impacts on benthic infauna and demersal fish. The study measured the extent and magnitude of macrobenthic community composition across the Southern California Bight and characterized the trends in that condition over the last 15 years (Bight'13 CIAC 2013).

Documentation of project management, design, and methods for the Southern California Bight Regional Monitoring Program 2013 survey are contained in the following documents[5]:

- Southern California Bight 2013 Regional Marine Monitoring Survey (Bight'13): Contaminant Impact Assessment Workplan (Bight'13 CIAC 2013)
- Southern California Bight 2013 Regional Marine Monitoring Survey (Bight'13): Contaminant Impact Assessment Field Operations Manual (Bight'13 FSLC 2013)
- Southern California Bight 2013 Regional Marine Monitoring Survey (Bight'13): Macrobenthic (Infaunal) Sample Analysis Laboratory Manual (Bight'13 Benthic Committee 2013)
- Southern California Bight 2013 Regional Monitoring Survey (Bight'13): Information Management Plan (Bight'13 IMC 2013)
- Southern California Bight 2013 Regional Monitoring Program: Volume VI. Benthic Infauna (Gillett et al. 2017)
- Sediment Quality Assessment Technical Support Manual (Bay et al. 2014)

The Bight'13 study used a Generalized Random Tessellated (hexagonal grid) Stratified sampling design developed by EMAP, with the strata corresponding to the subpopulations of interest. Sites were selected randomly within strata, to ensure that they were representative and could be extrapolated to the response of the entire strata. A systematic component was added

[5] Available at http://www.sccwrp.org/Documents/BightDocuments/Bight13Documents.aspx.

to the selection process to minimize clustering of sample sites (Bight'13 CIAC 2013, Bight'13 FSLC 2013).

Sediment chemistry samples including sediment grain size, TOC, total organic nitrogen (TON), and contaminants were collected with a 0.1- $m^2$ Van Veen grab. The top 2 cm of the undisturbed surface material at the offshore sites and the top 5 cm at the inner coastal stations (bays, harbors, estuaries, etc.) were removed from the grab. Sediment in contact with or within 1 cm of the metal sides of the grab was avoided to prevent sample contamination (Bight'13 FSLC 2013). Section VIII: Benthic Sampling in the Bight'13 Contaminant Impact Assessment Field Operations Manual (Bight'13 FSLC 2013) and the Sediment Quality Assessment Technical Support Manual (Bay et al. 2014) contain procedures for sediment chemistry sample collection, processing, quality control, and storage.

Benthic macrofauna samples were collected following a random tessellation stratified design from 12 different strata at 361 sites across the Southern California Bight ranging from Point Conception in the north to the US-Mexico border in the south. Samples were collected from July through September with a 0.1- $m^2$ Van Veen grab, sieved on a 1-mm screen, and then preserved for identification to the lowest possible taxonomic level (Bight'13 FSLC 2013, Gillett et al. 2017). Section VIII within Bight'13 FSLC (2013) contains grab sampling procedures, criteria for acceptable grab samples, required sampling event data, and sample processing. Macrobenthic sample analysis procedures are detailed in the Bight'13 Macrobenthic (Infaunal) Sample Analysis Laboratory Manual (Bight'13 Benthic Committee 2013) including sample treatment and storage, sample sorting, taxonomic analysis, data submission, and quality control.

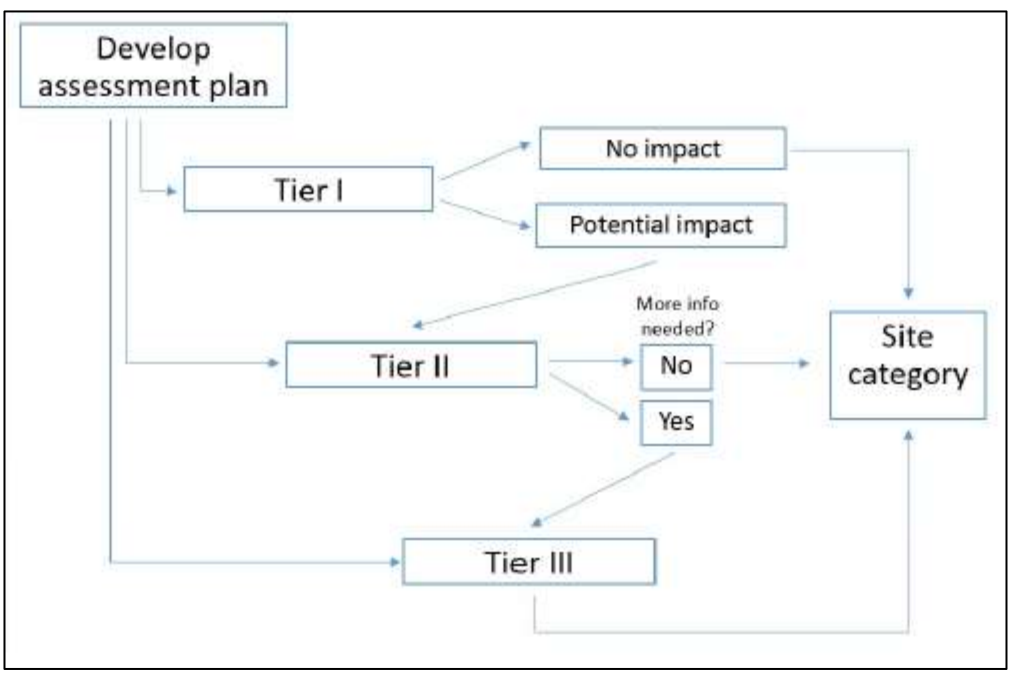
Macrobenthic community composition among the different strata was evaluated using MDS ordination of Bray-Curtis similarity values of all sites (Gillette et al. 2017). Community analyses were done with the metaMDS (similarity and ordination) and envFit (species and environmental factor correlations) programs within the R Vegan package (R version 3.2.5) or Primer v6 (SIMPER analysis; Gillette et al. 2017). The California Sediment Quality Objectives Benthic Line of Evidence (SQO BLOE) framework was used to assess samples from embayments (Gillett et al. 2017). The SQO BLOE is a combination of four indices: two multi-metric indices (Index of Biotic Integrity [IBI] and Relative Benthic Index [RBI]), a BRI abundance weighted tolerance index, and an Observed:Expected (O:E) index. The four SQO BLOE are scored and integrated into four condition categories functionally equivalent to those of the Smith et al. (2001) BRI following Ranasinghe et al. (2012a; Gillett et al. 2017). Temporal trends in habitat condition were calculated with two complementary techniques: a multi-survey approach and a revisit-site approach. The multi-survey approach is a higher-level approach to temporal analysis that focused on the proportional change in each of the condition categories across the whole of the survey area through time (Gillett et al. 2017). The revisit sites approach provided a more coarse measure of condition change by focusing solely on the temporal variance (Gillett et al. 2017).

Several special studies are also mentioned in the Bight'13 Contaminant Impact Assessment Field Operations Manual including: toxicity test on the polychaete *Neanthes*, Toxicity Identification Evaluations (TIE) testing, chemicals of emerging concern, DNA preservation of benthic samples, and bioaccumulation in infauna animals (Bight'13 FSLC 2013).

### 3.6.2 *Common Approach to Assess Sediment Quality*

SCCWRP is developing a common approach for assessing sediment quality and for assisting managers in translating sediment science into decisions (SCCWRP 2015). This approach is contained in the Southern California Coastal Water Research Project Authority, Thematic Research Plan for Sediment Quality referenced as SCCWRP (2015). The plan assesses impacts from direct exposure of contaminants to benthic organisms using multiple lines of evidence (MLOE), as no single line of evidence can definitively measure impacts of sediment contamination, and each line of evidence has limitations. The recommended MLOE are sediment chemistry, sediment toxicity, and biological assessment (impacts to benthic macrofauna communities). The report also contains potential new research and methods for habitat quality assessment. One important accomplishment was the indirect assessment conceptual framework that consists of three tiers of analysis to address variations in site complexity and data availability that integrates two independent indicators to produce a categorical output describing the relative degree of impact associated with sediment contamination within a site (Figure 4; SCCWRP 2015).

**Figure 4. The three tiered indirect assessment conceptual framework model developed by SCCWRP (taken from SCCWRP 2015).**



**Summary** – Several aspects including: project organization, a random stratified sample design, the use of PRIMER software for benthic community analysis, the use of a combination of four benthic indices, MLOE, and the three tiered assessment conceptual framework could be useful for updating the MEP benthic infaunal survey. The SCCWRP benthic macrofauna sampling method indicates that the continued use of a modified Van Veen grab in August and September would be appropriate for an updated MEP benthic sampling procedure.

## 3.7 Published Literature

Our review of the published literature focused on sample collection methods, sieve mesh size, preservatives, and types of benthic habitat indicators. The most common collection methods used for assessing benthic habitat quality in intertidal and subtidal waters consisting of hard and soft bottom substrates are summarized in Table 2[6]. The table also provides type of species or data collected and whether data results will be qualitative or quantitative along with relative cost and assessment value based on our best professional judgement.

### *3.7.1 Sieve Mesh Size*

Several different sieve mesh sizes are used for the extraction of benthic infaunal organisms from sediments; for example, 300 µm (0.3 mm), 500 µm (0.5 mm), and 1.0 mm. The mesh size selected for a study defines the minimum size of the fauna collected. Benthic invertebrate body size varies with species and with life stage within each species. Benthic invertebrate species in soft-bottom habitats are grouped based on body size into different sub-components of the benthic community. Megafauna (greater than 1 cm), macrofauna (greater than 0.5 mm), meiofauna (less than 0.5 mm), and microfauna (less than 0.05 mm) are typically considered separately based on differing ecological roles and sample collection methodologies. Despite this classification, benthic studies are rarely designed to strictly delineate a particular component of the benthic community. For example, both epifauna and infauna are captured in grab samplers, and a 0.5-mm-mesh screen retains both megafauna and macrofauna, along with some meiofaunal organisms. Most surveys of soft-bottom benthos in Atlantic coastal waters have focused on macrofauna (Brooks et al. 2006).

The choice of the sieve mesh size used for a program depends on the particular aims of the study (e.g., a general characterization of infaunal assemblages or a study of recruitment), coarseness of the sediment (which affects the volume of sediment retained and hence processing time) and the desirability or not of collecting juvenile macrofauna (which can be difficult to identify since taxonomic descriptions are usually based on characteristics of adults; Maciolek-Blake et al. 1985, James et al. 1995, Gibson et al. 2000). The use of a larger mesh size (1.0 mm) is preferred when sediments contain detritus, as in wetlands or estuarine environments, to prevent clogging of the sieve (Tagliapietra and Sigovini 2010). A 1.0 mm mesh size is sufficient for descriptive surveys, biomass estimates, or bionomics studies (Bachelet 1990, Rumohr 2009). The use of a finer mesh size (≤ 0.5 mm) is recommended for special purposes,

[6] References used to generate Table 2 included Holme and McIntyre (1984), Murphy and Willis (1996), Gibson (2000), Zale et al. (2012), and Somerfield and Warwick (2013).

**Table 2. Common collection methods used for assessing benthic habitat quality in intertidal and subtidal waters with hard and soft bottom substrates (Holme and McIntyre 1984, Murphy and Willis 1996, Gibson 2000, Zale et al. 2012, and Somerfield and Warwick 2013).**

| Areas of Study | Habitat | Method | Target Species or Data Type Collected | Qualitative/ Quantitative | Analysis Results | Cost (Low =L, Medium =M, High =H) | Assessment Value (Low =L, Medium =M, High =H) |
|---|---|---|---|---|---|---|---|
| Intertidal | Hard Bottom/ Armored Surfaces | Transects | Encrusting, Fixed, Mobile Invertebrates | Quantitative | Species ID, abundance | L[1]/ M | M- invasive species presence = indicator |
| | Soft Bottom | Grabs/Cores | Sediment grain size, Sediment chemistry, Infaunal species | Quantitative | Species composition, abundance, diversity | M[2]/ H | H- using literature to determine stress tolerant/intolerant indicator species |
| | | Quadrats | Epifaunal and infaunal invertebrates, Sediment grain size, Sediment chemistry, Seagrasses or macroalgae | Quantitative[3] | Species composition, abundance, diversity | M[1]/ H | H- using literature to determine stress tolerant/intolerant indicator species |
| | | Transects | Epifaunal and infaunal invertebrates, Sediment grain size, Sediment chemistry, Seagrasses or macroalgae | Quantitative[3] | Species composition, abundance, diversity | M[1]/ H | H- using literature to determine stress tolerant/intolerant indicator species |
| Subtidal | Hard Bottom | Underwater Imagery | | | | | |
| | | HD Camera - Diver | Encrusting and mobile invertebrates, Demersal fish | Semi-quantitative | Species composition, relative abundance | H (diver and analysis cost) | L/M-unable to collect infaunal species |
| | | HD Camera - Drop Frame | Encrusting and mobile invertebrates, Demersal fish | Semi-quantitative | Species composition, relative abundance | H (analysis cost) | L/M-unable to collect infaunal species |
| | | Remotely Operated Underwater Vehicle (ROV) | Encrusting and mobile invertebrates, Demersal fish | Semi-quantitative | Species composition, relative abundance | H (equipment and analysis cost) | L/M-unable to collect infaunal species |
| | | Autonomous Underwater Vehicle (AUV) | Encrusting and mobile invertebrates, Demersal fish, Bottom habitats/ features | Semi-quantitative/ Qualitative[4] | Species composition, relative abundance | H (equipment and analysis cost) | L/M-unable to collect infaunal species |
| | | Quadrats/transects | | | | | |
| | | Diver | Encrusting and mobile invertebrates, Demersal fish, and Collection of sediment, epifauna, and infaunal samples | Quantitative | Species composition, abundance, diversity | H (diver cost) | H - collection of infauna species |
| | | ROV | Encrusting and mobile invertebrates, Sediment, Water quality | Quantitative | Species composition, abundance, diversity | H (equipment) | L/M-unable to collect infaunal species |
| | | AUV | Water quality, Bottom habitats/ features | Quantitative/ Qualitative[4] | Species composition, relative abundance | H (equipment and analysis cost) | L/M-unable to collect infaunal species |
| | | Traps | Mobile Invertebrates, Demersal Fish | Qualitative | Species occurrence | L | L - limited organisms collected |
| | | Suction/ Vacuum Samplers | Encrusting and mobile invertebrates - Collection of epifauna samples for ID | Quantitative | Species composition, abundance, diversity | H (diver cost) | H - collection of infauna species |
| | | Trawls | Demersal fish and invertebrates | Qualitative | Species occurrence | M | L - collection of inverts. limited |

**Table 2. Continued.**

| Areas of Study | Habitat | Method | Target Species or Data Type Collected | Qualitative/ Quantitative | Analysis Results | Cost (Low =L, Medium =M, High =H) | Assessment Value (Low =L, Medium =M, High =H) |
|---|---|---|---|---|---|---|---|
| Subtidal | Soft Bottom | Grabs/Cores | Sediment grain size, Sediment chemistry, Infaunal species | Quantitative | Species composition, abundance, diversity | H | H- using literature to determine stress tolerant/intolerant indicator species |
| | | Quadrats/Transects | | | | | |
| | | Diver | Sediment grain size, Sediment chemistry, Infaunal species | Quantitative | Species composition, abundance, diversity | H (diver cost) | H - collection of sediment and infauna species |
| | | ROV | Sediment grain size, Sediment chemistry, Infaunal species | Quantitative | Species composition, abundance, diversity | H (equipment) | H - collection of sediment and infauna species |
| | | AUV | Water quality, Bottom habitats/ features | Quantitative/ Qualitative[4] | Species composition, relative abundance | H (equipment and analysis cost) | M- data may need to be ground-truthed |
| | | Underwater imagery | | | | | |
| | | HD Camera - Diver | Encrusting and mobile invertebrates, Demersal fish | Semi-quantitative | Species composition, relative abundance | H (diver and analysis cost) | L/M-unable to collect infaunal species |
| | | HD Camera - Drop Frame | Encrusting and mobile invertebrates, Demersal fish | Semi-quantitative | Species composition, relative abundance | H (analysis cost) | L/M-unable to collect infaunal species |
| | | ROV | Encrusting and mobile invertebrates, Demersal fish | Semi-quantitative | Species composition, relative abundance | H (equipment) | L/M-unable to collect infaunal species |
| | | AUV | Encrusting and mobile invertebrates, Demersal fish, Bottom habitats/ features | Semi-quantitative/ Qualitative[4] | Species composition, relative abundance | H (equipment) | L/M-unable to collect infaunal species |
| | | Sediment Profile Images (SPI) | Habitat quality indicator - Depth and type of burrowing organisms | Quantitative | Archivable snapshot of biological, chemical, and physical processes occurring in the first few cm of the bottom sediment | H | H - qualitative habitat results |
| | | Trawls | Demersal fish and invertebrates | Qualitative | Species occurrence | M | L - collection of invertebrates limited |
| | | Traps | Mobile Invertebrates, Demersal Fish | Qualitative | Species occurrence | L | L - limited organisms collected |
| | | Suction/ vacuum samplers | Encrusting and mobile invertebrates - Collection of epifauna samples for ID | Quantitative | Species composition, abundance, diversity | H (diver cost) | H |

Notes:

[1]Volunteers could conduct intertidal transects and quadrats for a short list of selected species (e.g. invasive, commercially important, or macroalgae) on which they have been trained.

[2]Volunteers could collect intertidal soft substrate grab or core samples after they have received training.

[3]Observational information of invasive or rare species from citizens or volunteers would provide qualitative data of medium assessment value.

[4]Depending on the type of data collected by the AUV sensor array ground-truthing may be necessary, for example sediment type from sonar, providing qualitative data.

such as a detailed production study, in order to cover the entire size or age range of a population of interest (Rumohr 2009). Finer mesh sizes provide improved estimates of infaunal density and seasonal fluctuations at population and community levels (Maciolek-Blake et al. 1985). The main advantage of using finer mesh sizes is the retention of both large and small-bodied taxa, and juvenile and adult life stages of an organism (Gibson et al. 2000). Mesh sizes of 0.1 mm and 0.2 mm are suggested for use in macrofaunal population dynamics studies to provide adequate abundance estimates of individuals in small size classes (Bachelet 1990). The main disadvantage of using finer mesh sizes is the increased cost of sample processing. For example, using a 0.5-mm mesh rather than a 1.0-mm mesh could increase retention of total macrofaunal organisms by 130 to 180%; however, costs for sample processing may increase from approximately 35% to as much as 200% due primarily to sorting and taxonomic identification (US EPA 1986-1991, Gibson et al. 2000, Hartwell and Fukuyama 2015). If finer mesh sizes (≤ 0.5 mm) are used in addition to a 1.0 mm or 0.5 mm size, sieves should be nested and the sieve fractions treated separately throughout the laboratory and identification process with results given for the individual and summed fractions (Maciolek-Blake et al. 1985, Rumohr 2009). The use of different or unique mesh sizes can limit the comparability of results between studies and areas; as a result Gibson et al. (2000) recommended that a standard sieve mesh size be selected for all benthic monitoring programs. A review of the estuarine monitoring programs from around the nation showed the use of both 0.5 mm and 1.0 mm mesh sizes, with the majority of programs using a 0.5 mm mesh size (Gibson et al. 2000). In coastal waters, the 0.5 mm mesh size has generally become the standard for macrofaunal sampling (Gage et al. 2002). Comparisons among studies require careful attention to the details of sampling and processing methodology (Brook et al. 2006).

Several studies have examined the influence of different sieve mesh sizes on benthic infaunal collection and monitoring results (Maciolek-Blake et al. 1985, Bachelet 1990, James et al. 1995, Hilbig and Blake 2000, Gage et al. 2002, Hammerstrom et al. 2010, Hartwell and Fukuyama 2015). Generally, these studies found the 1.0 mm mesh had significantly lower sieving efficiency and retention rate compared to a 0.5 mm mesh (Bachelet 1990, Hammerstom et al. 2010, Hartwell and Fukuyama 2015). The studies showed that sieve mesh size had an effect on benthic descriptors including the number of species, abundance, diversity, evenness, and feeding guilds (Bachelet 1990, James et al. 1995, Hammerstrom et al. 2010, Hartwell and Fukuyama 2015). Mesh size efficiency, which affects taxonomic composition, shows considerable seasonal variation due to settlement pulses, and differs according to phyla and between species with in a phylum. Generally, crustacean abundance is the most affected followed by polychaete, and bivalve (Bachelet 1990, Hammerstom et al. 2010, Hartwell and Fukuyama 2015). The studies indicated variable results and the authors did not entirely agree on the magnitude of the impact of differences in sieving efficiency depending on which parameter was being examined (e.g. abundance, biomass, and diversity) and the statistical approach (Hartwell and Fukuyama 2015).

In addition to effects of sieve size, James et al. (1995) examined how the level of taxonomic resolution affects interpretations of spatial patterns in macrofaunal assemblages. Like sieve size, the taxonomic level of macrofaunal identification should depend on the aim of the study.

Several studies (Warwick 1988, Ferraro and Cole 1990, Warwick et al. 1990, Ferraro and Cole 1992) indicate that in some instances species-level taxonomic identification does not yield any more information than family- or even phylum-level identification (Gibson et al. 2000). Patterns of spatial variation consistent with anthropogenic disturbances are often similar both for species and broader taxonomic classifications of macrofauna and meiofauna (James et al. 1995). The degree of taxonomic resolution required to adequately characterize the community depends on the diversity present in the community. While species level identification is desired for macroinvertebrates surveys, it is often costly and assessment needs can generally be met at the genus level for classifying sites as minimally impaired or impaired (Gibson et al. 2000). However, species-level identification may be required to assess the sources of impairment (Gibson et al. 2000). James et al. (1995) suggest that if a choice needs to be made between larger mesh-size (e.g. 1.0 mm) or coarser taxonomic resolution, it is important to consider that if samples are processed at a coarse taxonomic level and stored, further analyses could be done later at a finer taxonomic resolution if necessary (e.g. a particular species is later shown to be environmentally sensitive). It is not possible, however, to go back and redo sampling with a finer sieve mesh.

Two sieve mesh sizes, 0.5 mm and 0.3 mm, have been used in benthic monitoring programs in Massachusetts and the New England region. Programs by the Massachusetts Office of Coastal Zone Management and the National Coastal Condition Assessment used the 0.5 mm sieve (Normandeau 2010, AECOM 2012, US EPA 2015a, Hubbard 2016). The Massachusetts Estuaries Project (MEP) benthic infauna sampling and the Massachusetts Water Resources Authority (MWRA) Benthic Monitoring Program use the 0.3 mm sieve (Howes and Samimy 2003, Rutecki et al. 2017). The MWRA program selected the unusually fine 0.3 mm mesh sieve in the development of the Secondary Treatment Facilities Plan (1987–1988) and the current long-term benthic monitoring programs in Boston Harbor and Massachusetts Bay to ensure regional consistency in the offshore New England benthic data (Hilbig and Blake 2000). Consequently, MWRA adopted the same techniques used for the benthic infaunal studies conducted on Georges Bank in 1980–1982 (Grassle et al. 1985) and the Georges Bank Benthic Infauna Monitoring Program (1981–1985; Maciolek-Blake et al. 1985; Hilbig and Blake 2000). Hilbig and Blake (2000) note that the comparison of MWRA results to those from other areas where different methodologies were used should be done with caution.

### *3.7.2 Preservation*

A number of substances and preservation procedures have been described in the literature for the different taxonomic groups that occur in benthic macrofaunal assemblages (Lincoln and Sheals 1979, Brusca 1980, Pollock 1998). The most abundant groups in these assemblages are often polychaetes, crustaceans, and mollusks (de Souza and Barros 2017). The two most commonly used sample preservation procedures are 1) fixation in 4-10% formalin with subsequent preservation in 70% ethanol, and 2) preservation in 70% ethanol without the use of a fixative substance (Eleftheriou and Holme 1984, de Souza and Barros 2017). Fixation is the hardening of tissues to retain their original shape, while preservation is the long-term storage of specimens (Pollock 1998). The most common fixative is 10% formalin; however its use requires some precautions (Pollock 1998; de Souza and Barros 2017). Formalin is acidic and requires buffering with borax or hexamine to prevent damage to animals with calcareous parts, therefore formalin is not considered a good long-term storage medium these organisms (Pollock 1998). Formalin generally fixes organisms in 48 hours (Lincoln and Sheals 1979). Secondly, formalin is a toxic compound with carcinogenic effects that requires protective equipment for eyes and hands and should be worked with in a well-ventilated area (Pollock 1998, de Souza and Barros 2017). Disposal of this material must follow federal, state, and local regulations. Due the precautions with formalin mentioned above, some researchers prefer to use only ethanol or reagent alcohol to preserve samples (de Souza and Barros 2017). However there are some disadvantages to using ethanol in the field including is volatility, precipitate formation when ethanol is mixed with seawater, shrinkage of soft-bodied organisms (desiccation of tissues), loss of organism weight (desiccation of tissues), separation of lamellibranchs from their shells, and loss of specimen color (Lincoln and Sheals 1979, Mills et al. 1982, Eleftheriou and Moore 2013, de Souza and Barros 2017). Ethanol does not penetrate tissues as well as formalin; as a result organisms preserved without formalin-fixing can become soft. Polychaetes showed a better degree of conservation (more rigid and intact structures) when fixed in formalin compared to those only preserved with 70% ethanol, and showed a significantly higher abundance with formalin-fixing in sandy sediment (de Souza and Barros 2017).

### *3.7.3 Benthic Indicators and Indices*

There is a large amount of published literature on benthic indicators and indices for determining habitat quality. Diaz et al. (2004), Salas et al. (2006), and Spilmont (2013) discuss a variety of existing benthic indicators and indices along with some of the considerations for their use. Our review focused on literature where the authors specified the indices were applicable to or modified for estuaries which have variable conditions and salinity gradients. We focused on studies and indices that occurred in or were developed for the Virginian Biogeographic Province, the Gulf of Maine, the United States Atlantic east coast, and other areas of the United States but did not exclude studies from other regions or countries that seemed useful due to similarities in estuarine habitats and the ability to detect anthropogenic stressors. A list of some of the publications reviewed discussing existing benthic indices is presented in Appendix A.

Borja et al. (2011) provides a categorized list of indices for assessing environmental quality based on the structure of macroinvertebrate communities in estuaries and lagoons (transitional waters; Figure 5). A number of approaches have been used to create the existing benthic indices (Diaz et al. 2004, Borja and Dauer 2008, Ranasinghe et al. 2009). Several of these indices such as Weisberg et al. (1997), Van Dolah et al. (1999), Paul et al. (2001), and Smith et al. (2001) are used in the programs summarized above and discussed in the publications presented in Appendix A. Diaz et al. (2004) evaluated 64 indices designed for use in aquatic habitats in the United States, Europe, and other regions. Salas et al. (2006) provides an overview of 33 indices and discusses on how to select the most suitable ecological indicators and index taking into account the type of disturbance, the type of community, and the data available. Current benthic indictors have some drawbacks including expert dependence, methodological dependence, and temporal variability. Most of the indicators are specific to a habitat or geographical area and are highly

**Figure 5. List of indices for assessing environmental quality in transitional waters (taken from Borja et al. 2011).**

**Table 5** Indices for assessing environmental quality based on the structure of macroinvertebrates in transitional waters

Univariate indices

*Descriptors*

Number of species (species richness), abundance (A), biomass (B)

*Indices of diversity*

- Shannon–Wiener diversity index (H') (Shannon and Weaver, 1949)
- Simpson's indices of dominance, diversity and evenness (Simpson, 1949)
- Brillouin indices of diversity and evenness (Brillouin, 1956)
- Pielou evenness index (J') (Pielou, 1966)
- Margalef's index (Margalef, 1968)
- Hurlbert index (Hurlbert, 1971)
- Hill's diversity numbers and evenness measures (Hill, 1973)
- BPI: Benthic pollution index (Leppäkoski, 1975).
- Taxonomic diversity index and Taxonomic distinctness (Warwick and Clarke, 1995)

*Graphical methods*

- RFD: Rank-frequency diagram (Frontier, 1977)
- K-dominance curves (Lambshead et al., 1983)
- ABC curves (Warwick and Clarke, 1994).

*Ecological groups*

- Indice Annélidien de Pollution (Bellan, 1980)
- Biotic index (Hily, 1984)
- MMI: Macrofauna monitoring index (Roberts et al., 1998)
- AMBI: AZTI's marine biotic index (Borja et al., 2000)
- BENTIX (Simboura and Zenetos, 2002)
- ISI: Indicator species index (Rygg, 2002)
- IE2C: Indice Biotique et Indice d'Evaluation de l'Endofaune Côtière (Grall and Glémarec, 2003).
- BOPA: Benthic opportunistic polychaetes/amphipods ratio (Dauvin and Ruellet, 2007)
- BITS: Benthic index based on taxonomic sufficiency (Mistri and Munari, 2008)
- Benthic opportunistic annelids/amphipods ratio (Dauvin and Ruellet, 2009)

Functional indices

- ITI: Infaunal trophic index (Word, 1979)
- EQI: Ecofunctional quality index (Fano et al., 2003)

Multimetric indices

- Pollution coefficient (Satsmadjis, 1982)
- BQI: Biological quality index (Jeffrey et al., 1985)
- Organism sediment index (Rhoads and Germano, 1986)
- RTR: Infauna ratio-to-reference of sediment Quality Triad (Chapman et al., 1987)
- BIEC: Benthic index of estuarine condition (Weisberg et al., 1993)
- B-IBI: Benthic index of biotic integrity (Ranasinghe et al., 1994)
- BCI: Benthic condition index (Engle et al., 1994)
- BHQ: Benthic habitat quality (Nilsson and Rosenberg, 1997)
- VPBI: Virginia province benthic index (Paul et al., 2001)
- NQI: Norwegian quality index (Rygg, 2002)
- IEI: Index of environmental integrity (Paul, 2003)
- BQI: Benthic quality index (Rosenberg et al., 2004)
- IQI: Infaunal quality index (Prior et al., 2004)
- INES: Fuzzy index of environmental integrity for transitional environments (Mistri et al., 2005)
- MarBIT: Marine biotic index tool (Meyer et al., 2006)
- DKI: Danske Kvalitet Indeks (Borja et al., 2007)
- BEQI: Benthic ecosystem quality index (Van Hoey et al., 2007)
- BBI: Brackish water benthic index (Perus et al., 2007)
- DAPHNE (Forni and Occhipinti-Ambrogi, 2007)
- FINE: Fuzzy index of ecosystem integrity (Munari and Mistri, 2008)
- MISS: Macrobenthic index for semi-sheltered systems (Lavesque et al., 2009)

Multivariate approaches, packages and models

- PLI: Pollution load index (Jeffrey et al., 1985)
- BRI: Benthic response index (Smith et al., 2001)
- M-AMBI: Multivariate-AMBI (Borja et al., 2004a; Muxika et al., 2007)
- PRC: Principal response curves (Pardal et al., 2004)
- TICOR: Typology and reference conditions for portuguese and coastal waters (Bettencourt et al., 2004)
- Combination of indices: B-IBI & TICOR (Chainho et al., 2008)
- APBI: Acadian province benthic index (Hale and Helshe, 2008)
- P-BAT: Portuguese-benthic assessment tool (Pinto et al., 2009)

variable at seasonal and pluri-annual scales (Spilmont 2013). The use of a MLOE approach or a suite of multiple indices in assessing biological impacts is recommended and can overcome individual indictor or index drawbacks and the conflicting results from different indictors or indices (Dauer 1993, Salas et al. 2006, Ranasinghe et al. 2009). An example of using this approach in a large monitoring program is the SQO BLOE framework for embayment samples used in the Southern California Bight Regional Monitoring Program (see above).

Based on the literature reviewed the following indices, summarized in Table 3, could be used to determine benthic habitat quality in southeastern Massachusetts estuaries and warrant closer examination. These indices were selected for consideration based on 1) the index would perform in an estuary, 2) the index would apply to the southeastern Massachusetts region, and 3) the index tested for eutrophication and was successful at detecting a range of eutrophication levels. Most of the indices reviewed and summarized used sediment contaminants and/or metals analysis metric to determine ecological health, but did not specifically test for or mention eutrophication. It is unclear whether the indices that focused on contamination from metals and sediment toxicity would apply as well to eutrophication-based pollution, therefore only those indices that were specifically tested for eutrophication were recommended for use in the revised benthic monitoring.

- Margalef's Index ($D_{mg}$) is a simple species richness index that has good discriminant ability, and is found in many popular software packages (Magurran 2004, Gamito 2010). It is strongly dependent on sampling size and effort. Total number should be used when calculating Margalef's index to avoid sub-estimation (Gamito 2010). Salas et al. (2006) found that Margalef's index was one of the most successful measures in differentiating the diverse disturbance levels in organic enriched areas along the coasts of Portugal and Spain.
- Average taxonomic distinctness ($\Delta$+) and variation in taxonomic distinctness ($\Lambda$+) – are indices generated by the PRIMER software package. Tweedley et al. (2015) indicates that these indices have the potential to establish a baseline in a region that is entirely impacted to some degree, and where no reference sites are available. Additionally, these indices can be used for data consisting simply of species lists rather than quantitative measures of abundance. The authors determined that the taxonomic distinctness indices are considered appropriate indicators of anthropogenic disturbance in estuaries. However, Tweedley et al. (2015) used among other parameters, the aluminum (Al) level in the sediment samples as a reference or baseline to determine the health of the estuary, it is unclear if this method would apply to eutrophication due to its use of Al as a reference point.

  Total taxonomic distinctness (TTD) developed by Clarke and Warwick (2001) is the average taxonomic distinctness summed over all species of a community. TTD tracks closely to species richness, therefore it is only useful for tightly controlled designs in which effort is identical for the samples being compared or sampling is sufficiently comprehensive for the asymptote of the species-area curve to have been reached (Clarke and Warwick 2001). Salas et al. (2006) found that TTD was one of the most successful measures in differentiating the diverse disturbance levels in organic enriched areas along the coasts of Portugal and Spain. Schweiger et al. (2008) compared several commonly used phylogenetic indices to a set of requirements to provide guidelines for index selection. The authors recommended that, depending on the research question, TTD be used in addition to species richness in studies that compare interdependent communities where changes occur more gradually by species extinction or introduction.

- Acadian Province Benthic Index (APBI) – Hale and Heltshe (2008) developed a benthic index for the nearshore Gulf of Maine including waters as far south as Cape Cod Bay. Logistic regression with candidate measures of benthic species diversity, pollution sensitivity/tolerance, and community composition were used to discriminate sites with high and low benthic environmental quality (BEQ). BEQ was based on sediment metal and organic contaminant concentrations, TOC, sediment toxicity, and bottom water dissolved oxygen levels. Ten of the 49 benthic metrics tested showed a strong ability to discriminate stations. A model using the Shannon-Wiener diversity measure, Rosenberg's species pollution tolerance measure, and the percent capitellid polychaetes (or percent *Capitella* spp.) strongly discriminated stations, with an area under the receiver operating characteristic (ROC) curve of 0.82 and a classification accuracy of 80%. An analysis of similarity test showed that the community composition of low BEQ stations was significantly different from high BEQ stations. The authors also developed several candidate benthic indices and tested them with independent data from Massachusetts Bay and Casco Bay to help select and validate the best index.

  Hale (2012) tested spatial patterns of subtidal benthic invertebrates and physical-chemical variables in the nearshore Gulf of Maine to calibrate the APBI and to evaluate classical biogeographic studies along the Gulf of Maine coast to provide information for ecosystem-based management. Environmental data (temperature, salinity, sediment percent silt-clay, depth) correlated well with the ordination of benthic relative abundance data. Temperature was the most important factor affecting broad species distribution patterns, followed by salinity. The study suggested that accuracy of benthic indices for the nearshore Gulf of Maine might be improved by taking biogeographical differences among subregions into account.

  The APBI is one of the most relevant indices based on geographic area, which could be an important factor if specific species are used as indicators. The index was able to detect the difference between high and low BEQ, and also performed well at detecting differences between stations indicating that it can identify differences at a relatively fine scale. The APBI was developed to work in the Acadian Province which contains deeper, colder, saltier, and better oxygenated areas than the areas used to develop the Virginian Province Benthic Index. The Virginia Province extends northward to the tip of Cape Cod as a result southeast Massachusetts estuaries are considered part of this province. However the boundary between the two provinces is dynamic and leaky with warmwater taxa extending northward into the Gulf of Maine and coldwater taxa extending south to Long Island Sound, as a result APBI may be appropriate for estuaries on Cape Cod, Martha's Vineyard, Nantucket, and for Buzzards Bay. Factors to take into account when employing this index include: 1) that it applies only to soft-bottom communities, 2) the applicability of the index in low salinity areas is currently unknown, and 3) the index was developed using summer data and the authors suggest that the effect of seasonality should be assessed although that it may not be important when the goal is to look for human-induced changes.

- B-IBI (Benthic Index of Biotic Integrity) and the modified B-IBI –is a multi-metric index that reflects the degree to which component measures of biological response deviate from values expected in habitats that show no evidence of anthropogenic stress (Van Dolah et al. 1999). The natural variations in these measures due to various environmental factors (e.g. salinity, latitude) are accounted for by defining habitat-specific reference conditions for each metric (Van Dolah et al. 1999). Weisberg et al. (1997) developed the B-IBI using data from five Chesapeake Bay sampling programs which included 17 metrics, focusing on salinity and substrate. This integrative index correctly distinguished stressed sites from reference sites 93% of the time, with the highest validation rates occurring in high salinity habitats. This index can be more easily exported to other study areas than some of the other integrative indices.

  Van Dolah et al. (1999) modified the B-IBI for use in southeastern US estuaries to create an index that was effective in discriminating between degraded and non-degraded sites in a variety of habitat types. They concluded that the index could be used as a biological tool for detecting signals of degraded sediment quality in southeastern estuaries.

  The high percentage rate for detecting stressed sites combined with the ability to be used in areas other than the Chesapeake Bay makes this index a strong candidate for use in an updated MEP method for benthic community analysis. A potential weakness in this index may be its relatively lower ability to detect stressed sites in waters with salinities of less than 18 parts per thousand.

- MAIA (Mid-Atlantic Integrated Assessment Program) – Llanso et al. (2002a and b) developed an index using the B-IBI approach to assess mid-Atlantic region benthic community condition in estuaries where salinity and sediment composition are major factors structuring infaunal assemblages. Although application of the index to low salinity habitats should be done with caution, the MAIA index appeared to be reliable with a high likelihood of correctly identifying both degraded and non-degraded conditions. The index was expected to be of great utility in regional assessments as a tool for evaluating the integrity of benthic assemblages and tracking their condition over time.

- VPBI (Virginian Province Benthic Index) – The Virginian Province encompasses the coastal waters from Cape Cod, Massachusetts to the mouth of Chesapeake Bay, Virginia. Paul et al (2001) developed the VPBI using 48 metrics including biodiversity, community condition, individual health, functional organization, and taxonomic composition. It correctly classified sites over the full range of salinity (tidal-fresh to marine waters) and across grain sizes (silt–clay to sand).

Pelletier et al. (2010) identified estuarine benthic invertebrates that could be used as indicator species to detect presence or absence of pollution in the Virginian Biogeographic Province. The study identified 37 pollution sensitive and 30 tolerant estuarine macroinvertebrate indicator taxa Province using data from the Environmental Monitoring and Assessment Program (EMAP) following a modified Smith et al. (2001) approach (Figures 6 and 7). They identified species occurring at the extreme ends of the pollution gradient that had low variance to determine species with narrow and specific environmental tolerances.

This index could be very useful for evaluating the benthic infaunal communities in southeast Massachusetts estuaries for several reasons: 1) southeast Massachusetts is the regional boundary specified for this index, 2) the ability to classify sites at any point in an estuary and across grain sizes, and 3) the indicator species reported by Pelletier (2010) could be directly applied to this index.

- AMBI (AZTI's Marine Biotic Index) – is an abundance-weighted, tolerance value index that assesses habitat condition based on the relative abundance of taxa (indicator species) in different tolerance value groups (ecological groups; Gillett et al. 2015, Pelletier et al. 2018). AMBI was designed to establish the ecological quality of European coasts and is one of the most frequently used indices in Europe (Borja et al. 2000, Muxika et al. 2005, Salas et al. 2006, Hutton et al. 2015, Pelletier et al. 2018). AMBI has been widely incorporated into plans for the implementation of the EU Water Framework Directive. The index examines the response of soft-bottom benthic communities to natural and human-induced disturbances in estuarine and coastal environments, integrating long-term environmental conditions (Borja et al. 2000). The ecological group values are treated as pan-global and applicable to any coastal ecosystem (Gillett et al. 2015). The index is popular because it responds to human pressures, does not require extensive calibration and validation datasets, and uses a generalized conceptual reference definition (Pelletier et al. 2018). It is supplied as user-friendly freely available software that includes continuously updated species list with approximately 8,400 taxa from all seas (updated June 2017; AMBI v 5; Borja et al. 2012). Salas et al. (2006) showed that AMBI was able to distinguish between areas with organic enrichment, dredging activities, and less disturbance. They concluded that AMBI was a good tool for detecting pollution. Several authors have published variants of AMBI (e.g., BENTIX, MEDOCC) to address discrepancies in tolerance groups assignment and difference in the disturbance gradient compared to the theoretical model (Pelletier et al. 2018). AMBI has been applied or evaluated in number of regions outside of Europe including the United States, Canada, and South America (Muniz et al. 2005, Teixeira et al. 2012, Gillett et al. 2015, Hutton et al. 2015). These authors have shown that AMBI performance can be improved by using tolerance values tailored to the local setting (Pelletier et al. 2018). AMBI performance is less robust when there are low numbers of individuals and species present in a sample, as occurs in low salinity areas of an estuary (Gillett et al. 2015, Pelletier et al. 2018). Muxika et al. (2007) addressed this problem by combining AMBI scores with habitat measurement of species richness and diversity to produce a multivariate AMBI (M-AMBI).

Initial applications of AMBI in US waters by Borja et al. (2008a) and Teixeira et al. (2012) produced moderate to poor agreement with pre-existing locally calibrated indices, dissatisfaction with index performance, and recommendations of modifications to enhance AMBI performance including the use of a M-AMBI approach (Gillett et al. 2015). Gillett et al. (2015) modified and expanded the ecological group classifications of AMBI to create an integrated list of benthic species found along the three marine regions of the US coast (US AMBI). The expanded listed used to calculate AMBI for US waters improved the performance of the index. It was able to differentiate habitat condition from the three different US regions and correlated well with existing local indices from these areas. However, US AMBI compressed scores towards a moderate condition and was biased with salinity which resulted in the misclassification of reference sites as degraded in oligohaline and tidal freshwater habitats (Gillett et al. 2015). Pelletier et al. (2018) addressed these issues and developed US-M-AMBI by adapting the M-AMBI framework of Muxika et al. (2007) for the US coast using the Gillett et al. (2015) ecological group species list for the high and bad habitat thresholds needed for the M-AMBI algorithm. US-M-AMBI removed the compression response relative to local index response and the low salinity bias producing a better performing index that can be used in a wider variety of estuarine habitats. US-M-AMBI appears to be an appropriate index for comparing condition of US estuarine and coastal waters across a broad continental scale while providing the precision and accuracy of a locally developed index. It can provide managers with a tool to interpret local conditions in a national context (Pelletier et al. 2018). The index was developed to provide a nationwide indicator for the NCCA.

**Table 3. List of indices selected for consideration to determine eutrophication in southeastern Massachusetts estuaries.**

| Index | Reference | Region | Stressor (Nitrogen/Sewage or Contaminants/Metals) | No. of Stations (Replicates) | Useful to MEP | Not Useful to MEP |
|---|---|---|---|---|---|---|
| Margalef's Index | Salas et al 2006 | Europe/ Mediterranean | Organic enrichment | NA | Effective in detecting organic enrichment in areas along the coasts of Portugal and Spain.<br>It is found in many popular software packages. | The index is strongly dependent on sampling size and effort.<br>It is not as commonly used as other diversity measures. |
| Taxonomic distinctness indices (Δ+ and Λ+) | Tweedley et al. 2015 | Europe | Metals | 67(5) | Potential to establish a baseline in a region that is entirely impacted to some degree, and where no reference sites are available.<br>Can be used for data consisting simply of species lists rather than quantitative measures of abundance. | Study measured health of estuary by level of heavy metal. It is not clear if this method would work for organic enrichment since method included use of aluminum in the sediment to provide a reference or baseline. |
| | Warwick and Clarke (1998, 2001) in Salas et al. 2006 | Europe/ Mediterranean | Organic enrichment | NA | Total Taxonomic Distinctness (TTD) was able to distinguish between more and less organically enriched areas. | TTD tracks closely to species richness and is only useful for tightly controlled designs in which effort is identical for the samples being compared. |

**Table 3. Continued.**

| Index | Reference | Region | Stressor (Nitrogen/Sewage or Contaminants/Metals) | No. of Stations (Replicates) | Useful to MEP | Not Useful to MEP |
|---|---|---|---|---|---|---|
| Acadian Province Benthic Index (APBI) | Hale and Heltshe 2008 | Gulf of Maine (ME to Cape Cod Bay) | Metals and Organic Contaminants | 182 | Index Region includes Cape Cod Bay. | Although index region includes Cape Cod Bay, the conditions in the Gulf of Maine may not apply to those on the south side of and south of Cape Cod.<br>It is not clear if the index will be able to detect degrees of eutrophication as part of its ecological status. |
| B-IBI for mid-Atlantic (MAIA) | Weisberg et al. 1997 | Chesapeake Bay | Pollution. Includes response to organic enrichment | 2,319 | The simple additive scaling and equal weighting of metrics in the B-IBI allow users to examine its component parts easily and to identify how each metric contributes to the overall score. | Van Dolah et al. (1999) indicate that the normalization process can be complex and produce results that may not always be consistent with established ecological principals.<br>Classification efficiency was generally poor in low salinities. |
| | Van Dolah et al. 1999: Modified B-IBI (Weisberg et al 1997 for Chesapeake Bay) | VA, NC, SC, GA, FL | Contaminants/ sediment toxicity | 171 (2-4; mostly 3) | Criteria for classifying stations as reference or degraded.<br>Combining the index with other measures of habitat quality can improve assessment of the overall condition of a site or estuary. | The index was effective at the regional scale (Carolinian Province), but it is not clear how it will perform in the Virginian Biogeographic Province. |

**Table 3. Continued.**

| Index | Reference | Region | Stressor (Nitrogen/Sewage or Contaminants/Metals) | No. of Stations (Replicates) | Useful to MEP | Not Useful to MEP |
|---|---|---|---|---|---|---|
| MAIA Continued | Llanso et al. 2002a,b | Mid-Atlantic US | Contaminants | 1,999 | Index is reliable with a high likelihood of correctly identifying both degraded and non-degraded conditions.<br>It is expected to evaluate the integrity of benthic assemblages and track their condition over time. | It is not clear if the index will be able to detect eutrophication.<br>Salas et al. (2004) indicated that B-IBI was designed for very specific geographic areas in North America. This index is adapted for the mid-Atlantic; the species list may not apply to southeastern Massachusetts. |
| Virginian Province Benthic Index (VPBI) | Paul et al. 2001 | Cape Cod to the mouth of Chesapeake Bay, VI | Contaminants | Variable studies | Index region includes Cape Cod and Buzzards Bay.<br>It is able to classify sites at any point in an estuary and across grain sizes. | It is not clear if the index will be able to detect eutrophication. |
| | Pelletier et al. 2010 | Cape Cod to the mouth of Chesapeake Bay, VI | Pollution | 1,856 | Pollution tolerant and sensitive species apply to southeast Massachusetts.<br>Can be used on smaller data sets, assuming that there are not major habitat differences among the samples. | Organisms in the low mesohaline habitat were likely more influenced by salinity than by pollution. Points out the importance of accounting for habitat variation before attempting to isolate pollution effects. |

**Table 3. Continued.**

| Index | Reference | Region | Stressor (Nitrogen/Sewage or Contaminants/Metals) | No. of Stations (Replicates) | Useful to MEP | Not Useful to MEP |
|---|---|---|---|---|---|---|
| AZTI's Marine Biotic Index (AMBI) and multivariate approach (M-AMBI) | Salas et al. 2004 | Portugal | Eutrophication | 14 | Several indicator species referenced are present in southeastern Massachusetts.<br>Index tested specifically on eutrophication and performed well. | |
| | Muxika et al. 2005 | Baltic Sea, North Sea and Bay of Biscay, and the Mediterranean Sea | Pollution and eutrophication | 9 | AMBI is able to respond successfully to very different environmental impact sources, including eutrophication processes (see Salas et al. 2004). | The competitive ability of the species classified as opportunistic species in the AMBI is probably not advantageous in organically poor and naturally– physically stressed environments, such as the inner part of estuaries (as outlined by Borja et al., 2004).<br>Geographic region may not be applicable. |

**Table 3. Continued.**

| Index | Reference | Region | Stressor (Nitrogen/Sewage or Contaminants/Metals) | No. of Stations (Replicates) | Useful to MEP | Not Useful to MEP |
|---|---|---|---|---|---|---|
| AMBI and M-AMBI Continued | Gillett et al. 2015 (US-AMBI) | US Mid Atlantic (MA), US Southeast estuaries (SE), Southern California Bight bays and estuaries (SC) | Diagnosing habitat condition | Three regional validation datasets: MA = 568 SE = 60 SC = 685 | Ecological group species list and value for approximately 1,300 taxa developed from the National Coastal Assessment datasets. Geographic region is applicable. | The index showed compressed scores towards a moderate condition and was biased with salinity which resulted in the misclassification of high quality sites as degraded in oligohaline and tidal freshwater habitats compares to locally calibrated indices. |
| | Pelletier et al. 2018 (US-M-AMBI) | US Atlantic, Gulf of Mexico, and Pacific coastal waters | Diagnosing habitat condition | 4,061 | US-M-AMBI was developed to assess US coastal waters and can be used in a wide variety of estuarine habitats. It provides the precision and accuracy of a locally developed index and can help managers to interpret local conditions in a national context. Geographic region is applicable. | Developed for soft bottom benthos and may not be applicable for hard bottom and riprap areas. |

## Figure 6. Pollution sensitive taxa identified for the Virginian Biogeographic Province (taken from Pelletier et al. 2010).

**Table 2**

Pollution sensitive taxon with habitat, taxonomic and feeding guild information. The hashed boxes indicate where the species was present in a given habitat (TF – tidal freshwater, O – oligohaline, HM – high mesohaline, P_M – polyhaline mud, P_S – polyhaline sand), but not identified as an indicator species. The solid boxes indicate where the species was present and identified as an indicator species.

| | | | TF | O | HM | P_M | P_S |
|---|---|---|---|---|---|---|---|
| Annelida: Clitellata | | | | | | | |
| *Tubificoides wasselli* | Tubificid worm | deposit feeder [a] | | | ■ | ▒ | ▒ |
| Annelida: Polychaeta | | | | | | | |
| *Aricidea suecica* | Paraonid worm | deposit feeder [b] | | | | ■ | ▒ |
| *Brania clavata-swedmarki* complex | Syllid worm | carnivore [a] | | | | ■ | ▒ |
| *Diplocirrus hirsutus* | Flabelligerid worm | deposit feeder [b] | | | | ■ | |
| *Dipolydora caulleryi* | Spionid worm | deposit and suspension feeder [b] | | | | ■ | ▒ |
| *Dipolydora socialis* | Spionid worm | deposit and suspension feeder [b] | | | | ■ | ▒ |
| *Levinsenia gracilis* | Paraonid worm | deposit feeder [b] | | | | ■ | ▒ |
| *Lumbrinerides acuta* | Lumbrinerid worm | omnivore [a, b] | | | | | ■ |
| *Lumbrineris hebes* | Lumbrinerid worm | omnivore [a, b] | | | | ■ | ▒ |
| *Lumbrineris verrilli* | Lumbrinerid worm | omnivore [a, b] | | | | ■ | ▒ |
| *Magelona papillicornis* | Magelonid worm | deposit feeder [c] | | | | | ■ |
| *Monticellina baptisteae-dorsobran* | Cirratulid worm | deposit feeder [b] | | | | ■ | ▒ |
| *Nephtys incisa* | Nephtyid worm | carnivore [b] | | | ▒ | ■ | ▒ |
| *Polycirrus haematodes* | Terrebellid worm | deposit feeder [b] | | | | ■ | |
| *Protodriloides chaetifer* | Protodrilid worm | deposit feeder [d] | | | | | ■ |
| Arthropoda | | | | | | | |
| *Acanthohaustorius intermedius* | Amphipod | suspension feeder [e] | | | | | ■ |
| *Acanthohaustorius millsi* | Amphipod | suspension feeder [e] | | | ■ | | ▒ |
| *Bezzia* | Biting midge | omnivore [a] | ■ | ▒ | | | |
| *Chiridotea coeca* | Isopod | omnivore [a] | | | | | ■ |
| *Chiridotea tuftsi* | Isopod | omnivore [a] | ■ | ■ | | | |
| *Cyathura burbancki* | Isopod | omnivore [a] | | | ■ | ▒ | ▒ |
| *Hargeria rapax* | Tanaid | suspension feeder [f] | | | ▒ | ■ | ▒ |
| *Leptocheirus* | Amphipod | suspension and deposit feeder [a] | ■ | ▒ | | ▒ | |
| *Monoculodes edwardsi* | Amphipod | suspension and deposit feeder [a] | ▒ | ■ | | ▒ | ▒ |
| *Parahaustorius* | Amphipod | suspension feeder [e] | | | | | ■ |
| *Polypedilum simulans* | Chironomid | omnivore [a] | ■ | | | | |
| *Protohaustorius deichmannae* | Amphipod | suspension feeder [e] | | | | | ■ |
| *Protohaustorius wigleyi* | Amphipod | suspension feeder [e] | | | | | ■ |
| *Stictochironomus* | Chironomid | omnivore [a] | ■ | | | | ■ |
| *Tanaissus psammophilus* | Tanaid | suspension feeder [f] | | | | | ■ |
| Echinodermata | | | | | | | |
| *Micropholis atra* | Brittle star | deposit feeder [g] | | | | ■ | ▒ |
| Mollusca: Bivalvia | | | | | | | |
| *Astarte undata* | Astarte | suspension feeder [h] | | | | ■ | ▒ |
| *Nucula annulata* | Nut clam | deposit feeder [h] | | | ▒ | ■ | ▒ |
| *Nucula proxima* | Nut clam | deposit feeder [a] | | | | ■ | ▒ |
| *Pythinella cuneata* | Montacutid | suspension feeder [h] | | | | ■ | |
| Molllusca: Gastropoda | | | | | | | |
| *Acteocina* | Bubble | carnivore [a] | | | | ■ | |
| Phoronida | | | | | | | |
| *Phoronis* | Phoronid | suspension feeder [i] | | | ■ | ▒ | ▒ |

[a] Chesapeake Bay Program (2000).
[b] Rouse and Pleijel (2001).
[c] Fauchald and Jumars (1979).
[d] Glasby and Fauchald (2003).
[e] Bousfield (1970).
[f] Brusca and Brusca (2003).
[g] Chesapeake Bay Program (1994).
[h] Ruppert et al. (2004).
[i] Lippson and Lippson (2006).

**Figure 7. Pollution tolerant taxa identified for the Virginian Biogeographic Province (taken from Pelletier et al. 2010).**

**Table 3**
Pollution tolerant taxon with habitat, taxonomic and feeding guild information. The hashed boxes indicate where the species was present in a given habitat (TF – tidal freshwater, O – oligohaline, HM – high mesohaline, P_M – polyhaline mud, P_S – polyhaline sand), but not identified as an indicator species. The solid boxes indicate where the species was present and identified as an indicator species.

| | | | TF | O | HM | P_M | P_S |
|---|---|---|---|---|---|---|---|
| Annelida: Clitellata | | | | | | | |
| *Haber speciosus* | Tubificid worm | deposit feeder [a] | ▨ | ■ | | | |
| *Limnodrilus* | Tubificid worm | deposit feeder [a] | ■ | ■ | | | |
| *Limnodrilus cervix* | Tubificid worm | deposit feeder [a] | ■ | | | | |
| *Limnodrilus hoffmeisteri* | Tubificid worm | deposit feeder [a] | ■ | ■ | | | |
| *Paranais* | Naidid worm | deposit feeder [a] | | ■ | | | |
| *Peloscolex heterochaetus* | Tubificid worm | deposit feeder [a] | ▨ | ▨ | ■ | ▨ | ■ |
| *Tubificoides* | Tubificid worm | deposit feeder [a] | | ▨ | ■ | ▨ | ▨ |
| Annelida: Polychaeta | | | | | | | |
| *Amphicteis floridus* | Ampharetid worm | deposit feeder [b] | ▨ | ▨ | ▨ | ■ | ▨ |
| *Boccardiella* | Spionid worm | deposit and suspension feeder [b] | | | | ■ | |
| *Boccardiella ligerica* | Spionid worm | deposit and suspension feeder [b] | ▨ | ▨ | ■ | | |
| *Capitella capitata* | Capitellid worm | deposit feeder [b] | | | | ■ | ▨ |
| *Hypaniola* | Ampharetid worm | deposit feeder [b] | | ■ | ▨ | ■ | |
| *Laeonereis culveri* | Nereid worm | deposit feeder [c] | | ■ | ▨ | ■ | ■ |
| *Manayunkia speciosa* | Sabellid worm | suspension feeder [a] | ■ | | | ■ | |
| *Marenzelleria viridis* | Spionid worm | deposit and suspension feeder [b] | ▨ | ▨ | ▨ | ■ | ▨ |
| *Mediomastus* | Capitellid worm | deposit feeder [b] | | | ▨ | ▨ | ■ |
| *Nephtys simoni* | Nephtyid worm | carnivore [b] | | | | | ■ |
| *Pherusa plumosa* | Flabelligerid worm | deposit feeder [b] | | | | ▨ | ■ |
| *Tharyx dorsobranchialis* | Cirratulid worm | deposit feeder [b] | | | | ▨ | ■ |
| *Tharyx killariensis* | Cirratulid worm | deposit feeder [b] | | | | ▨ | ■ |
| Arthropoda | | | | | | | |
| *Coelotanypus* | Chironomid | omnivore [a] | ▨ | ▨ | ■ | | |
| *Haplocytheridea setipunctata* | Ostracod | suspension and deposit feeder [d] | | | ■ | ▨ | ▨ |
| *Leptocheirus plumulosus* | Amphipod | suspension and deposit feeder [a] | ▨ | ■ | ■ | ■ | ▨ |
| *Tanypus* | Chironomid | omnivore [a] | ▨ | ■ | | | |
| *Tanypus neopunctipennis* | Chironomid | omnivore [a] | | ■ | | | |
| Mollusca: Bivalvia | | | | | | | |
| *Ennucula tenuis* | Nut clam | deposit feeder [e] | | | | | ■ |
| *Periploma margaritaceum* | Spoon clam | suspension feeder [f] | | | | | ■ |
| *Pisidium compressum* | Pea clam | suspension feeder [f] | ■ | | | | |
| Molllusca: Gastropoda | | | | | | | |
| *Acteocina oryza* | Bubble | carnivore [a] | | | | ▨ | ■ |
| *Caecum pulchellum* | Caecum | omnivore [f] | | | | | ■ |

[a]Chesapeake Bay Program (2000).
[b]Rouse and Pleijel (2001).
[c]Mazurkiewicz (1975).
[d]Brusca and Brusca (2003).
[e]Ruppert et al. (2004).
[f]Chesapeake Bay Program (1994).

# 4 Recommendations

The following are the recommendation for updating the marine benthic macrofaunal monitoring approach to support pre- and post TMDLs evaluations and habitat condition assessments. Overall, it is recommended that sampling and analytical methods for the revised marine benthic macrofaunal monitoring should be consistent with the methods and procedures used in the National Coastal Condition Assessment (NCCA; US EPA 2015a, 2016b) and that ecological classifications follow the Coastal and Marine Ecological Classification Standard (CMECS; FGDC 2012) where appropriate. Following these established protocols and standards will ensure the revised approach and methods are comparable to other federal and state programs currently being conducted in Massachusetts state waters and the New England region, and reflect the most up-to-date and accurate scientific practices for conducting benthic monitoring. Specific recommendations are divided into four general sections: sampling methods, analytical methods, approach, and program management. These sections are presented below.

## 4.1 Recommended Sampling Methods

Specific recommendations for sampling methods are made on key topics identified during the literature review and TAC discussions. The recommendations in this section will focus on collection of benthic infauna from soft substrates since this is the primary method under review and proposed in the approach described below. The following sampling equipment is recommended for use in the revised marine benthic macrofaunal monitoring protocol: a 0.04 $m^2$ Young-modified Van Veen grab sampler, the 0.5 mm sieve mesh size, and the use of formalin as the initial fixative. The recommendations are described in more detail below. Specific field methods will be described in separate stand-alone MEP Field Standard Operating Procedure (SOP; Sweeny and Rutecki 2019a) and QAPP (Rutecki and Nestler 2019) to establish data quality objectives, data management, and project oversight. The level of detail contained in these documents is recommended to be comparable to the documents referenced above for the NCCA, MWRA, and SCCWRP programs. Field collection methods for grab sampling and water quality measurements described in the MEP Field SOP and revised MEP QAPP is recommended to be consistent with the NCCA methods described in US EPA (2016a).

A 0.04 $m^2$ Young-modified Van Veen grab sampler is the equipment recommended to be used to collect benthic infauna and sediment samples. The use of this sampler is well-established and consistent with the NCCA method, the previous MEP benthic infaunal collection method, and MA CZM and MWRA benthic macroinvertebrate sampling programs. The Young-modified Van Veen grab is considered an efficient grab sampler for collecting soft sediments ranging from fine silt mud to firm sand at a single point in shallow (<30 m) to shelf (30 -200 m) waters. The grab is light weight (<100 kg) and deployable from small to medium sized vessels (Eleftheriou and Moore 2013). The 0.04 $m^2$ grab is manufactured by a number of vendors so it is readily available.

At least two benthic infaunal samples should be collected and processed from each sampling location to ensure the required precision for program objectives and for the data to be of sufficient quality to be used in management decisions. A third sample is suggested to be collected and archived in case one of the other two samples is damaged or if a sample fails quality control and assurance guidelines. Three factors affect the number of infaunal samples required for quantitative sampling: desired precision, the mean catch (mean number of organisms per sample), and the degree of clumping of the fauna being sampled. The required precision is the 95% confidence limits expressed as a fraction of the mean (or a %; Holmes and McIntyre 1984). The program requires a high level of precision (95-100%) which means low variability in sample collection and a high level of reproducibility of results for sample sorting and taxonomic identification. The mean number of organisms per sample in the samples previously collected for MEP assessments in Pleasant Bay ranged from 3 to 2,640. The benthic infauna species present in Massachusetts estuaries are expected to have random to some clumping distributions at most sampling locations. Based on this information, 1 sample per location would be adequate for areas with a high mean number of organisms per sample (50-1,000), however to ensure that areas with low mean number of organisms per sample (~5-10), which could represent impaired or degraded habitats, are sufficiently sampled a second sample is required. Collecting and processing 2 samples at each location allows for the required precision while keeping costs low by not requiring additional sampling that may or may not result in a higher precision. Additionally, scientific literature generally recommends that replicates should always be collected (Holmes and McIntyre 1984).

Benthic infaunal samples should to be processed using a 0.5 mm mesh size sieve (See Section 3.7.1 above). This mesh size will capture adult benthic macrofauna life stages resulting in the general characterization of the infaunal assemblages and determination of site-specific and overall embayment health. The use of the 0.5 mm mesh size is consistent with the majority of national and regional studies including the NCCA and MA CZM.

The recommended preservation procedure is 10% buffered formalin for a minimum of 48 hours with subsequent preservation in reagent alcohol within 7 days of collection (See Section 3.7.2 above). Formalin is recommended as the initial fixative, even with some of the handling considerations, to ensure that all organisms contained in the infaunal grab samples (especially polychaetes) are fixed enough to allow the highest degree of conservation (more rigid and intact structures). A high degree of conservation will help achieve the most accurate taxonomic identification (e.g. species level) possible for the samples collected. This preservation procedure is consistent with the NCCA.

## 4.2 Recommended Analytical Methods

The specific recommendations for analytical methods based on the literature review and TAC discussions are provided below. A number of parameters and indices have been recommended to analyze the benthic infaunal community data for temporal and spatial patterns and to determine overall embayment health. Specific laboratory methods will be described in a separate stand-alone MEP Laboratory SOP (Sweeny and Rutecki 2019b) and QAPP (Rutecki and Nestler 2019) to establish data quality objectives, data management, and project oversight. The level of detail contained in these documents should be comparable to the documents referenced above for the NCCA, MWRA, and SCCWRP programs. Taxonomic identification described in the MEP Laboratory SOP and QAPP is recommended to be consistent with the NCCA taxonomic identification procedures described in US EPA (2016b).

Benthic macrofaunal specimens collected in the monitoring program are recommended to be identified to the lowest practical taxonomic level. Species is the target level for all organisms with the following exceptions: meiofauna (due to being smaller than 0.5 mm); Oligochaeta (Class); Chironomidae (Family) in samples from marine, polyhaline and mesohaline regions; and nematodes which will not be counted for infaunal analysis. The species level identification was recommended to prevent the loss of information regarding negative indicator species, to provide as much data as possible on species present in an embayment, and to meet the required taxonomic criteria for use of the recommended benthic index, US-M-AMBI (see below). This species level identification is consistent with the NCCA taxonomic procedures (US EPA 2016b).

The community parameters recommended for benthic community analysis are the number of species, total abundance, Shannon-Wiener diversity index (H'), and Pielou's evenness (J'). These four parameters are widely used and consistent with the previous MEP infaunal analyses. The use of these parameters will allow comparisons to be made between the previous and future MEP benthic infaunal assessments. Additionally, Margalef's index ($D_{mg}$) and Total Taxonomic Distinctness (TTD) are being suggested to provide additional information on community diversity and to confirm that areas with organic enrichment have been correctly distinguished through the four parameters above. Both Margalef's index and TTD are being recommended due to their effectiveness in detecting organic enrichment and the ability to differentiate between more and less organically enriched areas. As Margalef's index and Total Taxonomic Distinctness have sensitivities to sampling effort and are modestly used they should be considered secondary indicators. Total number should be used when calculating Margalef's index to avoid sub-estimation (Gamito 2010).

US-M-AMBI is the recommended benthic index to determine site-specific and overall embayment benthic habitat health. M-AMBI integrates both the structural and functional aspects of the benthic community, and has been shown to have a high sensitivity to environmental variation from very different environmental impact sources, including eutrophication (Salas et al. 2004, Muxika et al. 2005, Hutton et al. 2015; See AMBI in Section 3.7.3 above). US-M-AMBI was developed specifically for the coastal regions of the United States to use in the NCCA. US-M-AMBI was designed to meet the need to assess US coastal waters at a continental-scale while providing the precision and accuracy of a locally-developed index. The use of US-M-AMBI in the MEP Benthic Monitoring Program will allow managers to interpret local conditions in a regional and national context.

Lastly, the complementary multivariate analyses of Bray-Curtis similarity hierarchical agglomerative clustering (cluster analysis) and non-metric multidimensional scaling (nMDS) are recommended to determine the spatial patterns in the overall similarity of benthic assemblages in an embayment. Cluster analysis produces a dendrogram that represents discrete groupings of samples along a scale of similarity. This representation is useful when delineating among sites with distinct community structure. nMDS produces an ordination plot in which the distance between samples represents their rank ordered similarities, with closer proximity in the plot representing higher similarity. Ordination provides a useful representation of patterns in community structure when assemblages vary along a steady gradation of differences among sites. The infaunal abundance data should be fourth-root transformed to ensure that all taxa, not just the numerical dominants, will contribute to similarity measures.

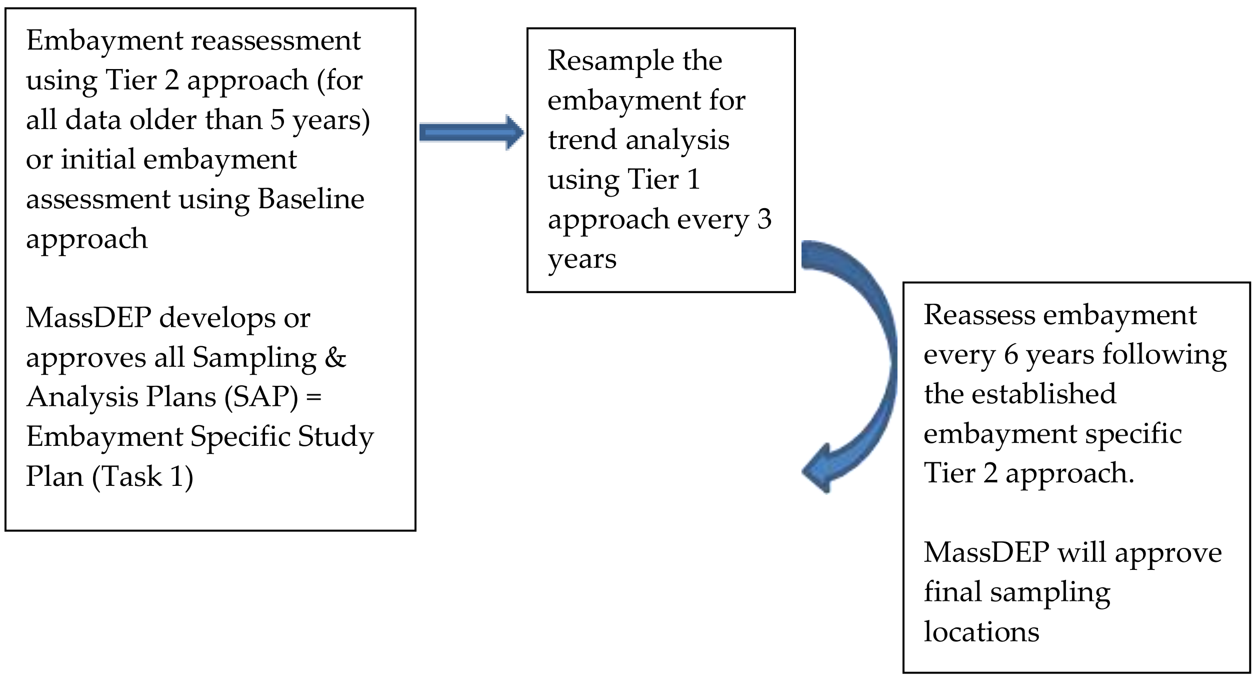
## 4.3 Recommended Approach

After a thorough review of the previous MEP documents, relevant regional and federal studies, the scientific literature and consultation with the TAC the following approaches are recommended for future baseline and post-TMDL implementation benthic monitoring. Two categories of approaches are recommended: 1) for embayments that were previously assessed by MEP, and 2) for embayments that have not been assessment for nutrient enrichment or overall embayment health. The previously assessed embayments have a two-tier approach that will provide useful information to managers and help make future decisions regarding the condition of the embayments. The difference in the tiers lies for the most part in sampling effort to help reduce the costs of long-term monitoring while providing the necessary information to make accurate assessments of embayment health. The previously assessed embayments have existing baseline information (e.g. sample locations and habitat health conditions); which will be used as appropriate for comparisons to future assessments with consultation from MassDEP. Unassessed embayments have a single baseline approach to collect the necessary data to make an initial assessment of embayment health. The initial (baseline) assessment will be used as the starting point for future assessments, and is similar to the Tier 2 approach for previously assessed embayments. Once an embayment is assessed future assessments will follow the two-tier approach. The conceptual framework for this benthic monitoring envisions the initial step of complete reassessment of a previously assessed embayment (when data is older than 5 years) or baseline assessment of an unassessed embayment followed by partial 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), starting the assessment cycle over again (Figure 8).

The sampling approach selected will be identified in an Embayment Specific Study Plan. 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 with field confirmation to allow for comparison between the initial and future assessments. GPS coordinates of the original benthic sampling locations are currently unavailable; the map in the technical report showing the benthic sample locations for the last embayment assessment will be overlaid into a GIS-software program (e.g. ArcView) to re-establish the locations using georeferencing techniques. During benthic sample collection, GPS coordinates will be taken to confirm these locations. The location and number of stations to be sampled for unassessed embayments will be determined using a Generalized Random

Tessellation Stratified (GRTS) survey design (See Section 4.3.1 below) for an area resource consistent with the NCCA. The development of the GRTS survey design will be done in consultation with MassDEP to ensure that enough samples will be collected to adequately assess the target population.

**Figure 8. Conceptual framework for the Massachusetts Estuaries Project Marine Benthic Monitoring Program.**




The TAC recommended the revised benthic monitoring include methods to characterize hard bottom benthic habitat (e.g. riprap) in embayments. Sampling methods for hard bottom and riprap areas were added to the benthic macrofaunal monitoring approaches along with another method as optional components. The three optional methods are: a stand-alone digital video benthic survey, Sediment Profile Imagery (SPI), and hard bottom /riprap destructive sampling (divers required for sample collection). These methods are recommended as optional components due to the relatively small size of the embayments under evaluation for pre- and post- TMDLs conditions, the required expertise to conduct the sampling, and the cost of deployment. The recommended marine benthic macrofaunal monitoring approach is embayment specific which will allow the use of the optional components in an individual estuary to address embayment specific concerns or unique habitats. The three methods are discussed briefly below.

- Stand-alone digital video benthic survey can be used to document the structure and appearance of embayment surface sediments, especially in areas with hard bottom substrates where other types of sampling are difficult. This method using a high-definition underwater camera via a diver or ROV can provide a visual record and information on the habitats present in the study area, species observed at the time of survey, evaluations of habitat use, observations of highly specialized behavior, species to species interactions, assessment of gear (e.g. fishing or aquaculture) performance or impact, and estimates of population size structure (e.g. shellfish beds). Digital video survey can provide data not available through other sampling techniques such as trawling or benthic grabs and is nonintrusive. The data collected is generally qualitative (descriptive).

- 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, as a result SPI is 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).

- Hard bottom /riprap destructive sampling is used to collect benthic macrofauna living on and/or in between rocks, boulders, submerged revetment (e.g. riprap and facility outfalls), and other hard substrates that cannot be sampled using a Van Veen grab sampler. The technique requires the use of scientific divers and a suction sampler to extract the organisms from the substrate. The collected organisms are identified to the lowest practical taxonomic level, usually species, and analyzed using the recommended community parameters and multivariate analyses (See Section 4.2). The method provides quantitative data that can be used in management decisions. Hard bottom /riprap destructive sampling is often used in conjunction with digital imaging to visually record the hard bottom features.

### *4.3.1 Generalized Random Tessellation Stratified Survey Design*

A GRTS spatial survey design is a representative sampling process that results in representative samples that are likely to be miniatures of the target population (Olsen et al. 2012). Natural resources (i.e. target population) occur as discrete objects (e.g. whole lakes), linear networks (e.g. stream), or collections of areas, represented in Geographic Information Systems (GIS) as collections of points (zero-dimensional objects), lines (1-dimensional objects), or polygons (two-dimensional objects; Stevens and Olsen 2004, Olsen et al. 2012). GRTS survey design has options for sampling these three types of populations. Additionally, the GRTS designs also include stratification, equal and unequal probability selection, and panel structure for surveys over time. A panel is defined as a collection of sites that have the same revisit pattern over time (Olsen et al. 2012). Software to implement GRTS survey design is available through the free software environment R project packages spsurvey. It is recommended that the GRTS survey design used for MEP marine benthic monitoring be determined in consultation with MassDEP.

For MEP marine benthic macrofaunal monitoring of a single embayment, the GRTS survey design for an area resource is recommended to be consistent with NCCA. An area resource is a continuous population that is present everywhere with a bounded area and does not have distinct natural units; therefore it is viewed as an infinite point set (Stevens and Olsen 2004). In general, a hierarchical square grid that covers the 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 benthic monitoring, the target population is the specific embayment selected for study generally following the defined NCCA estuarine resource area. Sites will be selected from all possible locations within the embayment subtidal benthic surface area. NCCA 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 body of coastal water. All waters within the enclosed area are defined as estuarine, regardless of depth or salinity (Olsen 2010, US EPA 2015b). It is recommended that the defined NCCA estuarine resource area be modified to include a depth requirement of 1 meter at mean low water for MEP benthic monitoring. This depth requirement will help ensure that only subtidal areas are included in the benthic monitoring target population and will excluded salt marshes.

The type of GRTS survey design selected (i.e. stratified or unstratified, and/or equal or unequal) for an embayment or program should be determined 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 (Stevens and Olsen 2003, Stevens and Olsen 2004, Kincaid 2018). The selection of a stratified or unstratified design will depend on the size and complexity of the embayment being studied. Stratification divides the sample frame into smaller separate sample frames that collectively equal the entire sample frame. Samples are selected from each stratum independently of the other stratum, applying the GRTS algorithm to each stratum (Olsen et al. 2012). For large or major embayments (e.g. Buzzards Bay, Mount Hope Bay, Cape Cod Bay, Massachusetts Bay) a stratified GRTS survey design is recommended. Parameters that could be used to develop defined strata in a GRTS survey design for MEP marine benthic macrofaunal monitoring include: depth at mean low water, bottom substrate type (if data available), salinity, and named estuary segment size. Water depths at mean low water could be obtained from NOAA nautical charts or an equivalent GIS data layer. Depth zones for a depth stratum are recommended to follow the CMECS benthic depth zone modifiers (Table 4). The littoral zone is defined as all areas that are episodically exposed to air. 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). Bottom type could include sediment grain size and habitat data from previous embayment studies, Massachusetts Shellfish Suitability Areas and DEP Eelgrass for Selected Embayments data layers (DMF 2011, MassDEP 2018), or equivalent GIS layers from other monitoring programs. Sediment grain size descriptors for a sediment stratum are recommended to follow the Wentworth (1922) standard for mineral grain size definitions as adapted by CMECS (Table 5). Salinity, if available, may include existing data and contour maps from previous studies or appropriate GIS layers. Salinity zones for a salinity stratum are recommended to follow the CMECS salinity modifiers (Table 6; FGDC 2012).

**Table 4. 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 |
| Mesobenthic | 200 to < 1,000 |

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

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

**Table 6. 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 |

The selection of an equal or unequal GRTS survey design will depend on the objectives of the study and whether specific sites within an embayment will be given sampling priority over other locations (Stevens and Olsen 2003, Stevens and Olsen 2004, Kincaid 2018). An unequal probability sample survey design is achieved by assigning a probability of selection to each category of the target population. In GRTS, an unequal probability sampling is implemented by giving each point (i.e. line-segment) a length proportional to its inclusion probability (Stevens and Olsen 2004, Olsen et al. 2012). Unlike a stratified sample, an unequal probability sample does not guarantee an exact number of sampling locations in each category, only that on average over repeated sample draws the selected probability for sampling locations will occur in each category with the total always being the total sample size (Olsen et al. 2012).

The GRTS survey design allows for the addition of units in a way that does not compromise the spatial balance and maximizes the overlap (co-location) of multiple studies such that all sample sizes are spread up (McDonald 2004). This flexibility allows GRTS to be combined with other designs in a mixed or hybrid design. Hybrid designs combine a fixed set of monitoring locations with additional random sites whose location are optimized to inform spatio-temporal processes (Lookingbill et al. 2012). Hybrid designs can be useful for either of the following conditions: 1) information is needed at multiple resolutions and/or multiple extents (e.g. inside and outside management unit boundaries), or 2) information is needed to respond to multiple management challenges (Lookingbill et al. 2012). It is recommended that MassDEP consider a mixed or hybrid design using GRTS for embayments or studies that contain multiple study objectives that cannot be fully attained using the designs discussed above, and for incorporating the GRTS survey design into previously assessed embayments that already have established sampling locations with baseline data.

### *4.3.2 Previously Assessed Embayments*

A summary of the sampling and analysis plans for Tiers 1 and 2 for southeast Massachusetts embayments that have been previously assessed is provided in Table 7.

**Tier 1:** The Tier 1 sampling plan is conducted 3 years after a full reassessment (Tier 2) or an initial assessment (Baseline). MassDEP will determine the sampling locations based on the results from the previous assessment giving priority to locations with the most impaired benthic habitats. The number of sampling locations can be up to 50% of the previously sampled benthic infaunal stations. Sampling at each location will consist of sediment grain size, TOC, and benthic infaunal sampling. Samples will be collected in August-October using a 0.04 $m^2$ Young-modified Van Veen grab sampler (See Section 4.1 above). Prior to grab sampling at least 2 digital images of the bottom surface and water quality measurements for temperature, dissolved oxygen, pH, and salinity should be recorded. Digital still images of the bottom collected using an inexpensive underwater digital camera (e.g. GoPro) will add little time or cost to the assessment, but provide valuable information regarding bottom sediment type, species interactions, and/or macroalgae presence. If the budget proves more limited than initially planned, the photos or footage can be archived and analyzed at a later date if warranted. Water quality measurements provide an understanding of the physical and chemical properties of the water column that affect benthic species and community occurrence and distribution in an embayment. Water quality measurements should be recorded using a multi-parameter water quality meter.

Benthic infaunal organisms collected during sampling will be identified to the lowest practical taxonomic level, usually species (See Section 4.2 above). Benthic infaunal data should be analyzed using all the recommended community parameters, multivariate analyses, and US-M-AMBI (See Section 4.2 above). Results from benthic infaunal analysis, sediment grain size, and TOC will be used to determine current habitat health condition. It is recommended to maintain the current MEP habitat health condition categories (i.e. 1) high quality habitat conditions, 2) moderately impaired, 3) significantly impaired, and 4) severely degraded [Howes et al. 2006a and b, Howes et al. 2013d]) at least initially to allow comparisons to be made to the baseline assessment health conditions in order to determine if there has been any change in embayment condition over time. It is also recommend that the US-M-AMBI conditions classes be used following Pelletier et al. (2018) to allow the incorporation of this index into the MEP monitoring program and to provide managers the ability to compare the health conditions of MEP estuaries in a regional and national context.

**Tier 2:** The Tier 2 sampling plan is conducted 3 years after a partial reassessment (Tier 1) or 6 or more years after the initial assessment (Baseline). MassDEP will determine the sampling locations based on the results from the previous assessment. The number of sampling locations can include all previously sampled benthic infaunal stations. If GPS coordinates of the original benthic sampling locations are currently unavailable, the map in the technical report showing the benthic sample locations for the last embayment assessment will be overlaid into a GIS-software program to re-establish the locations using georeferencing techniques. During benthic sample collection, GPS coordinates will be taken to confirm these locations. Sampling at each location will consist of sediment grain size, TOC, and benthic infaunal sampling. Samples will be collected in August-October using a 0.04 $m^2$ Young-modified Van Veen grab sampler (See Section 4.1 above). Prior to grab sampling at least 2 digital images of the bottom surface and water quality measurements for temperature, dissolved oxygen, pH, and salinity should be recorded. Digital still images of the bottom can be collected using an inexpensive underwater digital camera. If the budget proves more limited than initially planned, the photos or footage can be archived and analyzed at a later date if warranted. Water quality measurements should be recorded using a multi-parameter water quality meter.

Benthic infaunal organisms collected during sampling will be identified to the lowest practical taxonomic level, usually species (See Section 4.2 above). Benthic infaunal data should be analyzed using all the recommended community parameters, multivariate analyses, and US-M-AMBI (See Section 4.2 above). Results from benthic infaunal analysis, sediment grain size, and TOC will be used to determine current habitat health condition. It is recommended to maintain the current MEP habitat health condition categories (i.e. 1) high quality habitat conditions, 2) moderately impaired, 3) significantly impaired, and 4) severely degraded [Howes et al. 2006a and b, Howes et al. 2013d]) at least initially to allow comparisons to be made to the baseline assessment health conditions in order to determine if there has been any change in embayment condition over time. It is also recommend that the US-M-AMBI conditions classes be used following Pelletier et al. (2018) to allow the incorporation of this index into the MEP monitoring program and to provide managers the ability to compare the health conditions of MEP estuaries in a regional and national context.

**Table 7. Summary of recommended sampling and 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<br>Priority given to stations with most impaired benthic habitats<br>(Up to 50% of previously sampled benthic infaunal stations) | MassDEP to determine based on prior assessments<br>(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's index, and TTD.

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

### *4.3.3 Embayments Not Previously Assessed*

As mentioned above, there are many estuaries in southeastern Massachusetts that have no baseline data and habitat health conditions have not been assessed. The objective of the initial assessment is to obtain high quality data on the benthic habitat to determine the current health condition of the embayment in order to be used in management decisions (Table 8).

**Baseline:** The Baseline sampling plan includes the development of an embayment-specific sampling design (i.e. site selection and number of sites). The sampling locations will be determined using a GRTS survey design for an area resource (See Section 4.3.1). Software to implement GRTS survey design is available through the free software environment R project packages spsurvey. The GRTS survey design used for MEP marine benthic monitoring will be determined in consultation with MassDEP. MassDEP will approve all selected sampling locations.

Sediment grain size, TOC, and benthic infaunal samples will be collected in August-October at all of the sampling location identified in the embayment-specific study plan. Samples will be collected using a 0.04 $m^2$ Young-modified Van Veen grab sampler (See Section 4.1 above). Prior to grab sampling at least 2 digital images of the bottom surface and water quality measurements for temperature, dissolved oxygen, pH, and salinity should be recorded. Digital still images of the bottom can be collected using an inexpensive underwater digital camera. If the budget proves more limited than initially planned, the photos or footage can be archived and analyzed at a later date if warranted. Water quality measurements should be recorded using a multi-parameter water quality meter.

Benthic infaunal organisms collected during sampling will be identified to the lowest practical taxonomic level, usually species (See Section 4.2 above). Benthic infaunal data should be analysis using all the recommended community parameters, multivariate analyses, and US-M-AMBI (See Section 4.2 above). Results from benthic infaunal analysis, sediment grain size, and TOC will be used to determine current habitat health conditions. It is recommend that the US-M-AMBI conditions classes be used following Pelletier et al. (2018) to determine if there has been any change in embayment condition over time. This will also provide managers the ability to compare MEP estuaries' health conditions in a regional and national context. It is also recommended to maintain the current MEP habitat health condition categories (i.e. 1) high quality habitat conditions, 2) moderately impaired, 3) significantly impaired, and 4) severely degraded [Howes et al. 2006a and b, Howes et al. 2013d]) at least initially to allow comparisons to be made to other MEP estuaries that have not yet been reassessed using the US-M-AMBI index.

**Table 8. Summary of recommended sampling and analysis methods for southeastern Massachusetts embayments not previously assessed 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's index, and TTD.

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

## 4.4 Recommended Program Management

After reviewing documentation for the state, regional, and federal programs, it is recommended that the MWRA Ambient Monitoring Program be used as a template for project and data management with some modifications. This program's level of organization appears to be the most appropriate for the coordination between the towns of southeastern Massachusetts and the MassDEP MEP. An important feature of the MWRA Program is the direct oversight by the agency administrating the monitoring program ensuring access to complete data sets. Other programs such as the NCCA and SCCWRP were not selected due to the complexity of their program organization which is based on jurisdictional and regulatory agreements. Specifics for the project oversight and management roles (i.e. project coordination and interface with local authorities, project performance, and data management) should be described in separate stand-alone QAPP after further consultation with MassDEP.

Lastly, the revised and updated marine benthic macrofaunal monitoring approach described in this document can be expanded from the evaluation for pre- and post- TMDLs conditions in MEP embayments to an updated MassDEP marine benthic macrofaunal monitoring for all Massachusetts coastal waters. The use of a single benthic monitoring approach throughout coastal Massachusetts would ensure consistency and allow more accuracy within state comparisons of habitat conditions. The optional sampling methods discussed for rapid habitat assessment and hard bottom and riprap habitat types will allow the study of broader geographical areas and the additional habitat types found throughout Massachusetts coastal waters. The optional components also allow for individual embayments or studies to address specific questions or unique concerns while providing guidance to ensure data consistency and quality.

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# 6 Appendices

## 6.1 Appendix A. List of publications reviewed discussing existing benthic indices.

| Reference | Geographic Region | Index | Component of Benthic Community | Metric or Use | Study Conclusion or Comment |
|---|---|---|---|---|---|
| Bermejo et al. 2012 | Spain | Reduced species index (RSI) | Macroalgae | Species richness, proportion of red and green algae, ecological status group ratio, proportion of opportun. species | Significant correlation between water quality and index result (ecological status) |
| Bevilacqua et al. 2011 | Mediterranean Sea | Average taxonomic distinctiveness (Δ) Variation in taxonomic distinctiveness (Λ) | Molluscs and Polychaetes | Indices generated by Primer software | Indices showed higher sensitivity than classical indices in discerning between perturbed vs. unperturbed conditions (with some exceptions) |
| Blanchet et al. 2008 | France | Comparison of AMBI, BENTIX, BQI, Shannon-Wiener, BOPA | Benthic invertebrates | Assessing Ecological Quality Status | The 5 indices rarely agreed with each other, indicating that use of a single index would result in an inaccurate assessment |
| Boon et al. 2011 | Europe | Comparison of 22 common indices: AMBI, ITI, Shannon-Wiener, Margalef, DKI, NQI etc. | Benthos | Various | Results included ranking of best choices of indices for various applications; also details on decision tree for choice of metric/index |
| Borja & Dauer 2008 | General | Univariate: ABC, ITI, Sh-Wiener, EEI, BPI, taxonomic diversity and distinctiveness indices (Primer); Multivariate: AMBI, EQI, CoP, BQI, RTR, BEIC, BCI, B-IBI, Bentix; Multivariate methods decribing assemblage patterns: Benthic response index, estuarine trophic status, PRC, MDS, CANOCO, CDI | Benthos | Environmental quality in estuary | Introduction paper for special issue of Ecological Indicators |
| Borja et al. 2000 | Europe | Biotic Index (BI) using a biotic coefficient making it more usable for statistical analysis | Benthos | Various | Validation of BI is made with data from systems affected by human disturbances; thus anthropogenic changes in the environment can be detected |
| Borja et al. 2003 | Europe | Marine Biotic Coefficient (BC), AMBI | Soft-bottom Benthos | Aim: to assess BC usefulness in relation to different impact sources (e.g. heavy metals, submarine outfalls, industrial and mining wastes, jetties and sewerage works) | The results are consistent with using several other methods and parameters, such as richness, diversity, evenness, Abundance–Biomass comparison plots and univariate and multivariate statistical analyses. |
| Borja et al. 2008a | Chesapeake Bay | B-IBI, AMBI, M-AMBI | Benthos | Salinity regime | Similar results were seen for all 3 indices |

| Reference | Geographic Region | Index | Component of Benthic Community | Metric or Use | Study Conclusion or Comment |
|---|---|---|---|---|---|
| Borja et al. 2008b | Global/ North America | EPA NCA BI, (WQI), Sediment Quality (SQI), Benthic (BI), Coastal Habitat (CHI) | Benthos | Eutrophication | Reviews the current worldwide situation of integrative ecological assessment by presenting examples from Africa, Asia, Australia, Europe and North America. |
| Dauer 1993 | Chesapeake Bay | Model of community values: community biomass, # of individuals, species richness, % biomass of deep-dwelling species | Macrobenthos | Salinity | Model may be used as or to develop biological criteria for estuaries |
| Dauvin & Ruellet 2009 | France | Study proposes adaptation of BOPA to BO2A for use in freshwater transition zone | Benthos | | There is a need to adapt benthic indicators developed in coastal waters for transitional waters and to promote multi-criteria approaches. |
| Dauvin et al. 2010 | S. California | Benthic/biotic indices classified into 3 categories: 1) diversity – Margalef index, J' Pielou evenness index, H' Shannon-Wiener index, Simpson's Index, Benthic Quality Index (BQI); 2) ecological groups – AMBI, Ecological Quality Ratio (EQR), BENTIX, BOPA; and 3) trophic groups – ITI | Benthos | | Recommend pragmatism and thus the transfer of simple methods to the resarch consultancies that are responsible for assessing benthic quality in numerous impact studies. Using sentinel species, best professional judgement (BPJ), and taxonomic sufficiency is encouraged. |
| Dauvin et al. 2012 | Global | Comparison of Shannon-Wiener diversity, AMBI, BO2 A, and ITI | Benthos | | BPJ and opportunist sentinel species gave similar ECoQS for the different sampling sites. Discusses use of Biological Indicators as 'objective' or 'subjective' alternatives for assessing soft-bottom communities, and proposes BPJ and taxonomy sufficiency for diagnostic approaches. |
| Dauvin et al. 2016 | Northeast Atlantic and Mediterranean | Tested 3 indices: Benthic Opportunistic Annelids Amphipods (BO2A), Benthic Polychaete Opportunistic Families Amphipods (BPOFA),and EcoQs | Polychaetes and Amphipods | | EcoQs given by the BPOFA were very similar to those given by the BO2A. |
| Diaz et al. 2004 | East Coast US | 64 indices evaluated - summarized in tables | | Focus on habitat mapping | The leading edge of methods for benthic habitat mapping involves combining the advances in optical and acoustic methods that allow for routine classifying and mapping of the seafloor with biological and habitat data for species of concern. |

| Reference | Geographic Region | Index | Component of Benthic Community | Metric or Use | Study Conclusion or Comment |
|---|---|---|---|---|---|
| Fitch & Crowe 2010 | Ireland | M-AMBI, IQI, AMBI and ITI | Soft-sediment habitats | Investigated the sensitivity of communities, individual taxa, diversity indices and biotic indices. Nutrient and organic enrichment in intertidal soft-sediment habitats | M-AMBI and IQI, were more closely associated with nutrient and organic pollution than the AMBI and ITI indices |
| Goberville et al. 2011a | France | New mathematical procedures to evaluate the state of a system based on the relative reference state and indicators of nutrient over-enrichment | | SOMLIT software program | The multivariate procedures rapidly identified and evaluated anthropogenic nutrient anomalies from the continent on three sites |
| Goberville et al. 2011b | France | A new multivariate non-parametric procedure, based on the Mahalanobis generalised distance and a simplification of the multiple response permutation procedure to identify rapid changes in any natural systems. | | Procedure can be coupled on all monitoring programmes and is not influenced by missing data | The results indicate climate may interact with anthropogenic pressure to alter coastal marine systems and suggest a synergism between nutrient enrichment, human activities and local climatic conditions. |
| Guinda et al. 2008 | Spain | RSL and CFR | Macroalgae | | Results of applying both indices on three different types of pollution gradients off coastal Spain are presented. Generally, the CFR index responded more accurately than the RSL index to the pollution gradients under study. |
| Hale et al. 2012 | Nearshore Gulf of Maine | Multidimensional scaling done (MDS) on Bray-Curtis similarity matrices species relative abundance | Soft-bottom Communities | Aim: to provide information to calibrate benthic indices of ecological condition and to determine physical-chemical factors affecting species distributions of the Acadian Biogeographic Province | Accuracy of benthic indices for the nearshore Gulf of Maine might be improved by taking biogeographical differences among subregions into account. The results provide a foundation for ecosystem-based management, valuation of ecosystem services, conservation, and ocean spatial planning. |
| Hale and Heltshe 2008 | Nearshore Gulf of Maine | Multivariate benthic index for the nearshore Gulf of Maine | Soft-bottom Communities | 49 candidate measures of benthic species diversity, pollution sensitivity tolerance, and community composition to discriminate sites with high and low benthic environmental quality (BEQ). | 10 of the 49 benthic metrics showed a strong ability to discriminate stations. The applicability of the index in low salinity areas is unknown. The index works better for muddy areas than for sand. |

| Reference | Geographic Region | Index | Component of Benthic Community | Metric or Use | Study Conclusion or Comment |
|---|---|---|---|---|---|
| Halpern et al. 2008 | Global | Predicted cumulative impact scores (IC) | | Standardized, quantitative method used to estimate ecosystem-specific differences in impact of 17 anthropogenic drivers of ecological change | Study developed an ecosystem-specific, multiscale spatial model to synthesize 17 global data sets of anthropogenic drivers of ecological change for 20 marine ecosystems. |
| Hutton et al. 2015 | Montevideo estuary - South America | ITI, BENTIX, AMBI and M-AMBI | | Goal: test ITI, BENTIX, AMBI and M-AMBI for assessing the environmental quality of a coastal zone at a seasonal scale against many physicochemical variables. | From all the evaluated indices, AMBI appears to be the most suitable one to assess the environmental quality of the Montevideo coastal zone. AMBI was able to demonstrate spatial status gradients and shows a better correlation with contaminant levels in spite of the naturally stressed conditions of the estuary than the other indices. |
| Hyland et al. 2005 | 7 coastal regions of the world (including Boston Harbor and Mass. Bay) | Species richness, Hurlbert's Index E(Sn) | Macroinfaunal communities and total organic carbon (TOC) of sediment collected | | TOC critical points may be used as a general screening-level indicator for evaluating the likelihood of reduced sediment quality and associated bioeffects over broad coastal areas receiving organic wastes and other pollutants from human activities. |
| James et al. 1995 | Australia | MDS & ANOSIM (Primer) | | Goal: test whether coarser mesh-sizes and levels of taxonomic resolution will allow greater replication with little loss of information. | Mesh-size (0.5 mm or 1 mm) and taxonomic resolution (species or family) made little difference to the spatial patterns detected by non-parametric, multivariate analyses (MDS and ANOSIM) for assemblages of macrofauna but slightly more information was lost by using the coarser mesh than by using the coarser level of taxonomic resolution. |
| Karakassis et al. 2013 | Europe and Eastern Mediterranean | M-AMBI, BENTIX, BQI, Shannon Diversity index H', BOPA, BQI-family Index, BENTIX family index | | Assess indices along organic enrichment gradients. | Among the indicators tested, the BQI at the family level was the least sensitive in changes in the sampling configuration; it is highly correlated with all the other indicators and needs less time and taxonomic expertise. |
| Kroncke & Reiss 2010 | North Sea | AMBI, BOPA, M-AMBI, IQI, DKI and NQI | | Aim: Examine and compare the long-term variability of ecological indices | Univariate and most biotic and multimetric indices respond significantly on specific natural disturbance events such as cold winters, but the strength of response varied between indices as well as between events. |

| Reference | Geographic Region | Index | Component of Benthic Community | Metric or Use | Study Conclusion or Comment |
|---|---|---|---|---|---|
| Llanso et al. 2002a | Mid-Atlantic estuary; Delaware Bay through Pamlico Sound | Development of Mid-Atlantic Integrated Assessment Program (MAIA) | | Aim: develop an index for assessing benthic community condition in estuaries of the mid-Atlantic region. | Salinity and sediment composition were major factors structuring infaunal assemblages. Geographical location was a secondary factor. Differences between North Carolina and Delaware-Chesapeake Bay polyhaline assemblages were attributed to the relative contributions of species and not to differences in species composition. |
| Llanso et al. 2002b | Chesapeake Bay | Index was developed for the MAIA | | Aim: develop an index for assessing benthic community condition in estuaries of the mid-Atlantic region. | Although application of the index to low salinity habitats should be done with caution, the MAIA index appeared to be quite reliable with a high likelihood of correctly identifying both degraded and non-degraded conditions. The index was expected to be useful in regional assessments for evaluating the integrity of benthic assemblages and tracking their condition over time. |
| Miere & Dereu 1990 | Netherlands and Belgium | ABC | Intertidal Macrozoobenthos | | It is difficult to use this method in estuarine areas as an indicator of pollution because of the environmental stress typical for these areas. However, in general, it may be used to detect environmental stress. |
| Muxika et al. 2007 | Spain | AMBI | | Aim: to determine the minimal area and number of replicates necessary to obtain a precise estimate for AMBI | They determined that a minimal area of $0.25m^2$ was sufficient, for both intertidal and subtidal sampling stations, to classify 80% of the iterations into the same disturbance level and 2 replicates were sufficient to classify 80% of the pseudosamples into the same disturbance level, for 64% of the stations. |
| Neto et al. 2012 | Portugal | RSL | Macroalgae | | MarMAT was high inversely correlated with anthropogenic pressure and successfully reported all of the quality classes (bad to high) and captured the community changes more accurately when using the coverage of opportunists metric. MarMAT may be accepted as a compliant assessment methodology in the scope of the WFD requirements. |

| Reference | Geographic Region | Index | Component of Benthic Community | Metric or Use | Study Conclusion or Comment |
|---|---|---|---|---|---|
| Pagola-Carte et al. 2002 | Spain | Abundance, biomass and cover (ABC) | | Goal: Explore the relative effect on index results by earlier decisions concerning the type of measurement and the taxonomic resolution level. | The measurement type had a greater effect on the results than the taxonomic resolution used. Analyses based on abundance data usually lose more information when taxonomic resolution decreases than those based on biomass or cover estimates. |
| Paul et al. 2001 | Virginian Biogeographic Province (VBP) | Developed the Virginian Province Benthic Index (VPBI) | | 48 metrics including biodiversity, community condition, individual health, functional organization, and taxonomic composition. | Salinity was correlated significantly with some of the metrics and thus some metrics were normalized for salinity. Index correctly classified 87% of reference and 90% of degraded sites in the calibration data and 88% of reference and 81% of degraded sites in the validation data. It correctly classified sites over the full range of salinity (tidal-fresh to marine waters) and across grain sizes (silt–clay to sand). |
| Pelletier et al. 2010 | Virginian Biogeographic Province | Indicator species in the VBP | | Goal: identify estuarine benthic invertebrates that could be used as indicator species to detect presence or absence of pollution in the VBP | 67 taxa were identified as pollution indicator species; 37 pollution sensitive taxa and 30 pollution tolerant taxa. This technique can be used on smaller data sets, assuming that there are not major habitat differences among the samples, and can be applied to other coastal areas with mid-size (100–500 stations) monitoring data sets. |
| Pinto et al. 2009 | Portugal | Detailed Overview of APBI, AMBI ,M-AMBI, BENTIX, BHQ, BOPA, BQI, BRI, ISI, IEI, ITI, MMI, PLI, VPBI | | Tested different indices and combinations of indices in estuary | The use of several indices is always advised in order to get a better evaluation of the benthic community health and preferentially in association with other parameters. |
| Puente & Diaz 2008 | Spain | S, H, AMBI, M-AMBI, BQI, W-statistic, Taxonomic distinctness | | Tested the behavior and suitability of different biotic indices | Low species richness and dominance of a few tolerant species in the estuaries were challenging for biotic indices tested. Combined approaches that integrate different aspects of water quality and ecosystem functionality could increase the reliability of the ecological assessment of these transitional waters. |
| Ranasinghe et al. 2012b | Southern California | Southern California BRI and AMBI | Presence only data generated from DNA barcode | Evaluated the performance of BRI and the AMBI when species abundance data were removed from their calculation. | Associations between the presence and abundance BRI and the presence and abundance AMBI were highly significant. |

| Reference | Geographic Region | Index | Component of Benthic Community | Metric or Use | Study Conclusion or Comment |
|---|---|---|---|---|---|
| Reiss & Kroncke 2005 | Europe | Shannon-Weiner, ES100, AMBI, BQI, EQR | Macrofauna | Seasonal variability of several univariate and multimetric indices was studied on a monthly scale | The seasonal variability was highest for the univariate indices such as the Shannon–Wiener Index and the Hurlbert Index. Due to sensitivity to recruitment, ecological status ranged from good to poor depending on the season. Seasonal variability and the corresponding ecological status were low using multimetric indices (AMBI or the BQI). |
| Rosenberg et al. 2004 | Sweden | BQI | | Aim: develop a new method for classification of marine benthic quality according to the European Union Water Framework Directive. | Contains a short summary of some published work dealing with sensitive and tolerant marine benthic species. A BQI was calculated based on a combination of the species tolerance values, abundance, and diversity. |
| Rygg 2002 | Norway | ISI, ES100 | | Only presence/absence of the taxa (not abundance) is considered | Examples of the index are presented. |
| Salas et al. 2006 | Portugal and Spain | Table/key of 33 indices | | Key/table to help in the selection of the most suitable ecological indicators/index to use taking into account the type of disturbance and the data available. | Index selection depends on the type of disturbance or the level of taxonomic identification of the organisms |
| Sanchez-Moyano et al. 2006 | Spain | SIMPER (Primer) | | | Conclusion: different levels of taxonomic resolution (species, family, order) lead to similar results both with regard to relative community distributions and the environmental variables associated. The importance of this result for monitoring similar benthic communities is discussed. |
| Smith et al. 2001 | S. California | BRI | Coastal Shelf depth 10 - 324 m | Goal: present an objective quantitative index for shelf waters | |
| Tweedley et al. 2015 | UK | Number of taxa, overall density, Shannon–Wiener diversity, Simpson's index and AZTI's Marine Biotic Index [AMBI]), average taxonomic distinctness (Δ+) and variation in taxonomic distinctness (Λ+) | | | These taxonomic distinctness indices are considered appropriate indicators of anthropogenic disturbance in estuaries, as they allow a regional reference condition to be set from which significant departures can then be determined. |

| Reference | Geographic Region | Index | Component of Benthic Community | Metric or Use | Study Conclusion or Comment |
|---|---|---|---|---|---|
| Van Dolah et al. 1999 | southeast US | Modification of the B-IBI | | Goal: develop a benthic index for use in southeastern US estuaries effective in discriminating between degraded and non-degraded sites in a variety of habitat types. | Index can be used as a biological tool for detecting signals of degraded sediment quality in southeastern estuaries. |
| Ware et al. 2009 | UK | Number of species (S), number of individuals (N), average taxonomic, distinctness, taxonomic breadth and average phylogenetic diversity | | | (S) and (N) generally scored highest in terms of understandability, sensitivity and linkage to the human activity, whilst biotic indices were assigned relatively low scores, particularly in relation to aggregate extraction activities. |
| Weisberg et al. 1997 | Chesapeake Bay | B-IBI was developed using data from five Chesapeake Bay sampling programs | Salinity and substrate | 17 metrics | Index correctly distinguished stressed sites from reference sites 93% of the time, with the highest validation rates occurring in high salinity habitats. |

## 6.2 Appendix B. Literature Referenced in Figure 6 from Borja et al. 2011 Table 5.

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