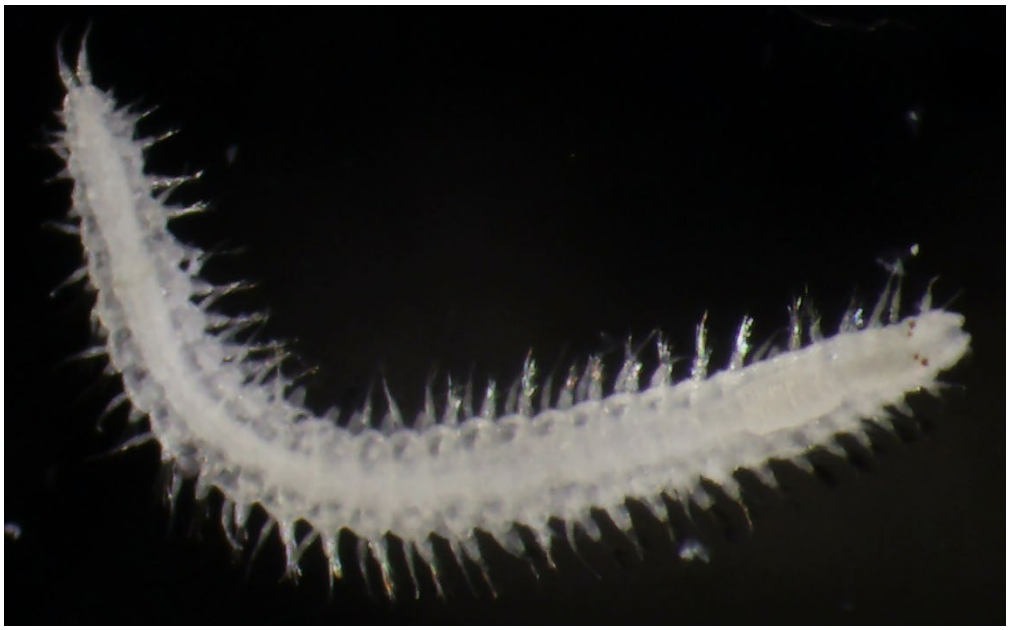
Benthic Monitoring Report:
Onset and Buttermilk Bay System 2023 Survey



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Polychaete *Salvatoria clavata*. Normandeau Associates, Inc.

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LIST OF ACRONYMS

ATD	Average Taxonomic Distinctiveness
AU	Assessment Unit
BI	Biological Index
BBC	Buzzards Bay Coalition
BBNEP	Buzzards Bay National Estuaries Program
cm	centimeter
CMECS	Coastal and Marine Ecological Classification Standard
CMR	Code of Massachusetts Regulations
CWMP	Comprehensive Wastewater Management Plan
D_{mg}	Margalef's diversity index
DO	dissolved oxygen
EG	Ecological Grouping
ft	feet
GRTS	Generalized Random Tessellation Stratified
H'	Shannon-Wiener diversity index
HD	High Definition
J'	Pielou's evenness index
m, m^2	meter, square meter
MA	Massachusetts
MA DMF or DMF	Massachusetts Division of Marine Fisheries
MassDEP	Massachusetts Department of Environmental Protection
MEP	Massachusetts Estuary Project
MDS	non-metric multidimensional scaling
mi, mi^2	mile, square mile
mg/L	milligrams per liter
mL	milliliter
µm	micrometer
N	abundance
NOAA	National Oceanic and Atmospheric Administration
OBB-2023	Onset and Buttermilk Bay System 2023 Survey
PRIMER	Plymouth Routines in Multivariate Ecological Research
PVC	polyvinyl chloride
QAPP	Quality Assurance Project Plan
QA/QC	Quality Assurance/Quality Control
1-λ'	Simpson diversity index
S	Species richness
SOP	Standard Operating Procedure
SWQS	Surface Water Quality Standards
TMDL	Total Maximum Daily Load
TOC	Total Organic Carbon
TWMP	Target Watershed Management Plan
US EPA	United States Environmental Protection Agency
USFWS	United States Fish and Wildlife Service
US M-AMBI	Multivariate AZTI Marine Biotic Index in United States coastal waters
WPP	Watershed Planning Program

1 Introduction

The Massachusetts Estuaries Project (MEP) was established in 2001 to monitor and protect estuarine ecosystems in southeastern Massachusetts embayments. The technical reports produced from these embayment assessments documented embayment specific baseline water quality, habitat health, and identified the actions required to restore nutrient impaired waters for approximately 70 embayments. MEP provided technical guidance in support of policies on nitrogen loading to embayments, wastewater management decisions, and establishment of nitrogen Total Maximum Daily Loads (TMDLs) for over 30 estuaries. Many communities have begun the process of integrated water resources management planning or have completed preparation of Comprehensive Wastewater Management Plans (CWMPs) or Targeted Watershed Management Plans (TWMPs).

MassDEP has generated guidance documents for the collection of post-TMDL implementation and future baseline MEP benthic monitoring data. The new guidance offers a tiered approach for previously assessed embayments and a baseline approach for unassessed embayments. The new guidance documents include a Marine Benthic Monitoring QAPP (MassDEP 2023a), which contains the Marine Benthic Monitoring Field Standard Operating Procedure (SOP) and Laboratory SOP (MassDEP 2023a Appendix A and B respectively). These were developed for future MEP benthic monitoring efforts to describe study objectives, field and laboratory techniques, data quality requirements and assessments, and data management.

The objectives of the MEP benthic monitoring program are to:

1. Reassess the ecological health of embayments previously assessed under the MEP. Embayment reassessment will confirm if ecosystem health in impaired areas has improved following the implementation of TMDLs and community measures as projected by the Linked Watershed-Embayment Model;

2. Evaluate the ecological health of southeastern Massachusetts embayments that have not been assessed. The data collected during an initial assessment will be used as a baseline to indicate current embayment health and to provide information for future management decisions; and

3. Determine if long-term changes are occurring in southeastern Massachusetts embayments that may indicate stress from eutrophication or other factors, including changes in species distribution, invasive species, and climate change.

The Onset and Buttermilk Bay System had not previously been assessed under the MEP and was selected by MassDEP to be assessed to inform management decisions and develop TMDLs. This report provides the water quality, sediment, and benthic results of the benthic monitoring conducted in the Onset and Buttermilk Bay System.

According to the Massachusetts Surface Water Quality Standards (SWQS; 314 Code of Massachusetts Regulations [CMR] 4.00[1], MassDEP 2021), all Onset and Buttermilk Bay System segments are Class SA,

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

Shellfishing waters (*see* 314 CMR 4.06(6)(b): Table 3: Buzzards Bay Coastal Drainage Area). These waters are designated as an excellent habitat for fish, other aquatic life and wildlife, for primary and secondary contact recreation, for shell harvesting without depuration, and shall have excellent aesthetic value.

Onset Bay, located in upper Buzzards Bay, comprises the outer bay and upper estuary which includes Broad Cove, Muddy Cove, and Shell Point Bay (Figure 1). The Bay is used for recreational boating with a Town Pier and several marinas, boat moorings and slips, four pump-out dock facilities and one pump-out boat, and swimming with six public beaches (Howes et al. 1999). Onset Bay is adjacent to the Cape Cod Canal and is within its mixing zone with waters from Cape Cod Bay (Howes et al. 1999). The Bay is flushed with low nutrient offshore waters from the Cape Cod Canal (Jakuba 2020), which can reach 4 knots (Howes et al. 1999). The flushing rate for Onset Bay is 1.2 days (residence time; Aubrey Consultants 1991). Overall, Onset Bay is shallow with depths ranging from 1.5 to 4.5 m mean lower low water (MLLW) with a navigable channel in the mid region of Onset Bay (Town of Wareham 2011). Onset Bay consists of a deeper central basin separated from the Hog Island Channel and Buzzards Bay by a shoal area near Onset Island (Louis Berger and Associates, Inc. 1996). There are no major river discharges in Onset Bay, resulting in high average salinities at the mouth (30.8 ppt) and the inland shallow coves (29.5 ppt; Howes et al. 1999).

Buttermilk Bay is a shallow, semi-enclosed coastal embayment to the east of Onset Bay and is connected on the inland portion through a narrow channel to Little Buttermilk Bay (Figure 1). Little Buttermilk Bay was initially a freshwater kettle pond adjacent to Buttermilk Bay and became connected with rising sea-level, but with restricted flushing (Howes et al. 1999). Tidal flow is solely from Buzzards Bay through Cohasset Narrows (Figure 1). The Bay has a mean depth of approximately 0.9 m and tidal range of 0.8 to 1.4 m (Valiela and Costa 1988). The flushing rate for Buttermilk Bay is 2.8 - 4.5 days (residence time; Tetratech 2023). Red Brook to the north, is the largest freshwater input into Buttermilk Bay (Valiela and Costa 1988). Salinity in Buttermilk Bay and Little Buttermilk Bay ranges from 11 ppt to 31 ppt (Valiela and Costa 1988). Salinities in the middle of Buttermilk Bay are somewhat fresher compared to the average salinity at the head of Buzzards Bay (30.9 ppt). The lowest salinities recorded in nearshore areas of Buttermilk Bay are due to stream and groundwater inputs (Valiela and Costa 1988).

The Buttermilk Bay System is used recreationally for swimming, water-skiing and other boating recreation. The area contains boat moorings and slips, and a marina providing a pump-out boat and dockside facility and a waste dump facility (Howes et al. 1999). Buttermilk Bay historically supported a robust shellfishery. However, harvest was significantly restricted in the 1980s due to bacterial contamination primarily from stormwater runoff (Howes et al. 1999). This impact resulted in a largescale remediation of all 30 stormwater discharges in Buttermilk Bay.

Sediment data for the Onset Bay and Buttermilk Bay are scarce. Studies indicate that Onset Harbor sediments are primarily sand (MDMF 2021) and sediments in Buttermilk Bay are also soft sand and fine sediments (Valiela et al 1991). This sediment characterization is supported by the shellfish suitability data that indicate nearly all of Onset Bay and Buttermilk Bay is a potential habitat for quahogs (*Mercenaria mercenaria*), which burrow in sandy to muddy sand bottoms (MassMapper 2022, Howes and Goehringer 1996). In addition to quahogs, Onset Bay contains potential habitat for bay scallops (*Argopecten irradians*), soft shell clams (*Mya arenaria*) limited to the shores, and American oysters (*Crassostrea virginica*) that are located in southeast Onset Bay (MassMapper 2022). Conditions for shellfish harvest in the Onset and Buttermilk Bay System are variable, with most of the system classified as 'Approved' or 'Conditionally Approved' (defined as '*closed some of the time due to rainfall or*

seasonally poor water quality or other predictable events. When open, it is treated as an Approved area'; Hickey et al. 2015).

Over the past 20 years, water quality conditions in the Onset and Buttermilk Bay System have improved sufficiently enough to allow development and survival of oysters and quahogs. The B-120 Shellfish Restoration Program successfully transplanted oysters and quahogs in Onset and Buttermilk Bay in response to the tank barge Bouchard No. 120 oil spill in 2003 (MDMF 2021a; see MassDEP 2023d). Lydia's Island off the northwest tip of Onset Bay was supplemented with single oysters yearly from 2017 through 2020. The three-year oyster restoration project is considered a success with a large and densely packed oyster bed currently classified as "Approved" for the direct harvest of shellfish throughout the year (MDMF 2021a). In addition, in 2018, 150,000 oysters were planted at two oyster reef sites in the Buttermilk Bay system, one within the town of Wareham (Buttermilk Bay) and one in Bourne (Little Buttermilk Bay) waters (MDMF 2021a). The presence of a large and densely packed oyster bed at Lydia's Island and out-planted oysters' growth recorded in Buttermilk Bay is believed to be evidence of successful three years of oyster restoration work at the site (MDMF 2021a).

Figure 1. The location of the Onset and Buttermilk Bay System, Massachusetts.

2 Methods

The Onset and Buttermilk Bay System 2023 Survey followed the methods outlined in the Massachusetts Estuaries Project Marine Benthic Monitoring Quality Assurance Project Plan (QAPP; MassDEP 2023a). It was comprised of four components: water quality measurement profiles, digital images, benthic infauna, and sediment conditions (grain size and total organic carbon [TOC]).

Detailed descriptions of the field and laboratory methods are in the MEP Benthic Monitoring QAPP, which includes the MEP Marine Benthic Monitoring Field SOP (MassDEP 2023a Appendix A), and the MEP Marine Benthic Monitoring Laboratory SOP (MassDEP 2023a Appendix B). A brief overview of the methods, focused on information specific to this survey, is provided below in Sections 2.1 to 2.3.

2.1 Field Methods

Water quality profiles, digital images, benthic infaunal, and sediment sampling was conducted at 14 Onset and Buttermilk Bay System stations identified in the Onset and Buttermilk Bay System Embayment-Specific Study Plan (MassDEP 2023d) on four days in October (3rd, 4th, 24th, and 31st; Table 1, Figure 2), 2023. All target and base stations were assessed as planned. A Garmin ECHOMAP UHD 64CV (accuracy +/-2 m) was used for navigation and to acquire coordinates at the location of each sample. Comparisons among sampling coordinates and target station locations confirmed that sampling was conducted within a 30 m target radius at each station.

Three infaunal grabs and one sediment grab were collected at each of the 14 stations with a 0.04m^2 Young-modified Van Veen grab sampler. One duplicate sediment grab was collected at Station MEP-SE-037 and MEP-SE-041 for quality control purposes, for a total of 42 infaunal and 32 sediment samples (16 grain size and 16 total organic carbon [TOC]). All infaunal samples were rinsed in the field with clean seawater through a 500-micrometer (µm) mesh sieve and fixed in 10% formalin in labelled jars. Samples were hand delivered to the Normandeau Falmouth, MA office; after seven days the samples were rinsed with fresh water and transferred to reagent alcohol for storage and transported to the Normandeau Bedford, NH laboratory for sorting and taxonomic identification. Samples for sediment grain size and TOC analysis were collected by scooping the surface sediment (0 to 2 cm) of each grab, homogenizing, and transferring approximately 50 mL to appropriate storage bags or jars. Sediment samples were hand delivered to the Normandeau Falmouth, MA office and immediately refrigerated. The samples were then transferred to Pace Analytical Laboratories within 24 hours by a Pace Alpha Analytical courier for grain size and TOC analysis.

Water quality measurement profiles were taken using an In-Situ Aqua TROLL 600 multi-parameter water quality sonde with data recorder. The following parameters were recorded: temperature, dissolved oxygen (DO), pH, and salinity/conductivity. The 0.1 m below the surface measurement was not recorded at the 14 stations, all other profile measurements were collected following the depths and protocol specified in the Marine Benthic Monitoring QAPP and Field SOP (MassDEP 2023a).

Digital video images for each sampling location were recorded using a Delta Vision Splashcam HD camera in a waterproof housing attached to a PVC frame designed to match the MassDEP eelgrass camera frame. The camera was set in a fixed position 1 m above the bottom (15.5 inches by 15.5 inches) with scaling lights set 4 inches apart. A GoPro Hero 3+ was also attached to the camera frame to provide digital still images and camera redundancy. Due to variable turbidity and shallow depths at some of the

stations, images used to visualize bottom sediments may vary in clarity and the scaling frame may not be visible.

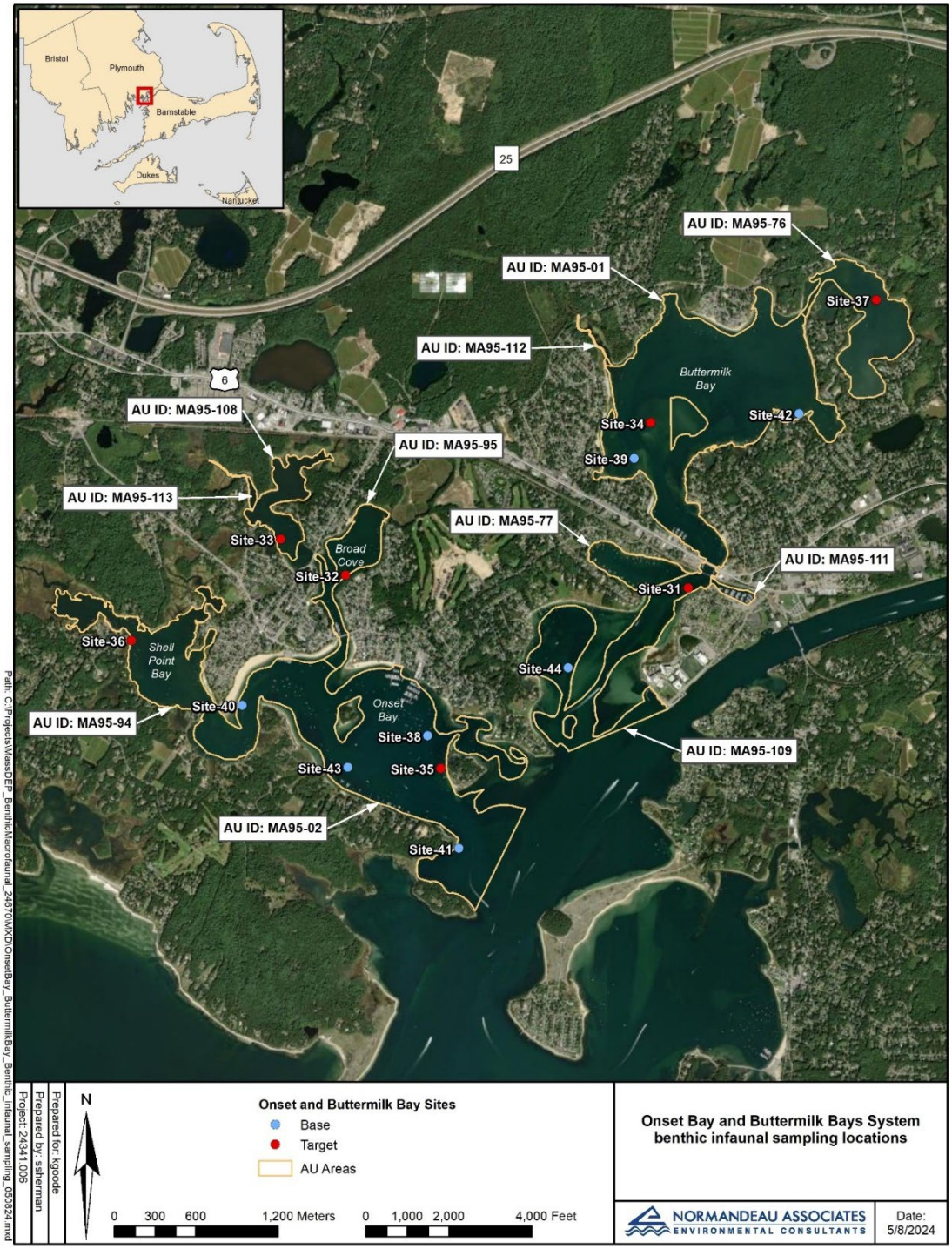


Figure 2. Benthic infaunal sampling locations in the Onset and Buttermilk Bay System.

Table 1. Listing of Preliminary Field Data from the Onset and Buttermilk Bay System 2023 Survey (OBB-2023).

Station[1]	Site Use	AU_ID	Subembayment	Date (time)	Depth (m)	Latitude[1]	Longitude	Comments
Onset Bay System								
MEP-SE-036	target	MA95-94	Shell Point Bay	10/3/2023 (1132)	1.0	41.74241	-70.67234	fine sediment
MEP-SE-033	target	MA95-108	Muddy Cove	10/31/2023 (1347)	1.3	41.74902	-70.65908	sandy
MEP-SE-032	target	MA95-95	East River/Broad Cove	10/3/2023 (1348)	1.8	41.74658	-70.65338	sulfur odor
MEP-SE-040	base	MA95-02	Onset Bay	10/3/2023 (1217)	2.4	41.73805	-70.66262	sandy
MEP-SE-043	base	MA95-02	Onset Bay	10/3/2023 (1254)	3.3	41.73387	-70.65332	fine sands and silt
MEP-SE-038	base	MA95-02	Onset Bay	10/3/2023 (1522)	3.3	41.7359	-70.64624	mud with seaweed, strong sulfur odor
MEP-SE-035	target	MA95-02	Onset Bay	10/4/2023 (1235)	2.4	41.73373	-70.64513	sandy with algae
MEP-SE-041	base	MA95-02	Onset Bay	10/4/2023 (1151)	2.4	41.72844	-70.64359	sandy silt
Buttermilk Bay System								
MEP-SE-037	target	MA 95-76	Little Buttermilk Bay	10/24/2023 (1054)	1.5	41.76443	-70.60621	silty sand
MEP-SE-042	base	MA95-01	Buttermilk Bay	10/24/2023 (1225)	0.5	41.75695	-70.61314	silty sand
MEP-SE-034	target	MA95-01	Buttermilk Bay	10/24/2023 (1336)	0.5	41.75646	-70.62626	silty sand
MEP-SE-039	base	MA95-01	Buttermilk Bay	10/24/2023 (1410)	0.7	41.75412	-70.62775	silty sand
MEP-SE-031	target	MA95-109	Cohasset Narrows	10/4/2023 (0956)	3.3	41.74552	-70.62311	coarse sand, rocky
MEP-SE-044	base	MA95-109	Cohasset Narrows	10/4/2023 (1105)	1.8	41.74032	-70.63377	silty sand, seaweed

[1]Sites are organized from innermost to outermost locations within the Onset and Buttermilk Bay Systems; see Figure 2.
[2]Latitude and longitude coordinates are in decimal degrees

2.2 Laboratory Methods

Laboratory methods were consistent with the MEP Marine Benthic Monitoring Laboratory SOP (MassDEP 2023a Appendix B). Two infaunal samples from each station were randomly selected for processing, while the third was archived. A total of 28 benthic samples from the Onset and Buttermilk Bay System were sorted, and 14 samples were archived. Organisms were sorted and identified to the lowest possible taxonomic level using a dissecting microscope. Each new distinct taxon was saved separately in a labeled vial with reagent alcohol and archived in a reference collection as directed under Section B4.1 of the MEP Benthic Monitoring QAPP.

Grain size samples were analyzed by Pace Analytical Laboratories following Section III of the MEP Marine Benthic Monitoring Laboratory SOP, using the ASTM Method D6913/D7928. One sediment sample (50 mL total volume) from each station, plus one additional sample each from MEP-SE-037 and MEP-SE-041 for quality control (QC) purposes, for a total of 16 samples were analyzed in the laboratory.

The analytical laboratory reported grain size in the Unified Soil Classification System. Grain size results were converted to the Coastal and Marine Ecological Classification Standard (CMECS) mineral grain size descriptors adopted from Wentworth (1922; FGDC 2012) using sieve size and the Folk (1974) conversion table. Grain size results were reported as a percentage by weight in five categories as follows:

- gravel = 2 mm to < 4,096 mm
- coarse sand = 500 µ to < 2 mm (includes the very coarse sand fraction)
- medium sand = 250 µ to < 500 µ
- very fine sand = 63 µ to < 250 µ (includes the fine sand fraction)
- silt = <63 µ

Marine and estuarine sediments generally consist of a mixture of grain sizes. For example, silty sand is defined as the combination of the three smallest sediment size classifications: fine sand, very fine sand, and silt.

Sediment samples for TOC followed the MEP Benthic Monitoring QAPP (MassDEP 2023a; Appendix A) for preservation and hold times. Analytical methods for TOC followed the US EPA Method 9060 (US EPA 2021).

2.3 Data Analysis

Benthic infauna data were analyzed for the following community parameters: abundance, Shannon-Wiener diversity index (H'), Pielou's evenness (J'), Margalef's species richness index (D_{mg}), Simpson, and Average Taxonomic Distinctiveness (ATD), using the PRIMER v7 (Plymouth Routines in Multivariate Ecological Research) software program (Warwick and Clarke 1991, Clarke and Gorley 2001). Shannon-Weiner (H') was calculated using natural log data.

Multivariate analyses were performed using PRIMER v7 software to examine spatial patterns in the overall similarity of benthic assemblages in the Onset and Buttermilk Bay System (Clarke 1993, Clarke and Warwick 2001). These analyses included classification (cluster analysis) by hierarchical agglomerative clustering with group average linking and ordination by non-metric multidimensional scaling (MDS). Bray-Curtis similarity was used as the basis for both classification and ordination. Similarity measures compare counts within each taxon between all possible pairs of samples. Values

range from 0, when two samples have no taxa in common, to 100 when two samples are identical in taxa and counts within taxa. MDS outputs a two-dimensional plot where spatial proximity illustrates relative similarity between samples and is interpreted by the closeness of the samples. Clarke (1993) suggested that a stress level less than 0.20 (shown in the upper right corner of the plot) indicates that a potentially useful two-dimensional representation has been achieved. The results are also presented with a hierarchical clustering tree diagram (a dendrogram), with the x-axis representing the full set of samples, and the y-axis defining a similarity level at which two samples or groups are considered to have fused (Clarke and Warwick 2001). To reduce the influence of high-density outliers, densities were square-root transformed before calculating similarity. The square-root transformation decreases the influence of the most abundant species so that rare species factor in more heavily when calculating similarity.

US M-AMBI (multivariate AZTI Marine Biotic Index in United States coastal waters) was calculated following Pelletier et al. (2018) to determine Onset and Buttermilk sub-embayment and embayment soft bottom habitat health. Modifications to the existing M-AMBI taxonomic classification (Ecological Grouping [EG]) were made prior to using the program utilizing the taxonomic list and corresponding EGs established by Pelletier et al. (2018) to be specific for the northeast US region. Each taxon identified was classified as EG I, II, III, IV, or V. Taxa categorized as I were considered those found in healthy benthic habitats, and V taxa inhabiting low quality habitat. The available published EG taxonomic list is for European studies, and some classifications are not the same as those for other regions. The revised taxonomic EG list specific to the northeast US region was provided by M. Pelletier (personal communication 2024). In this updated EG list oligochaetes are assigned an EG code of V, which is different from previous US M-AMBI calculations in which oligochaetes were not included in the calculation. This change was recommended by M. Pelletier based on updated information (personal communication 2024).

The data were prepared for US M-AMBI by first coding each station by salinity categories as defined by Pelletier et al. (2010): tidal fresh (< 0.5 ppt), low mesohaline (≥ 5 – 12 ppt), high mesohaline (≥ 12 – 18 ppt), and polyhaline (≥ 18 ppt) and then assigning each taxon with the Northeast United States EG codes (categories I-V). Some taxa in the Onset and Buttermilk Bay System samples were not included in the data set because no EG code was available for this region at this time (i.e., polychaete *Palposyllis prosostoma* and anemone *Nematostella vectensis*, etc.), or the specimens were not able to be identified to a low enough taxonomic level (i.e. Gastropoda and Bivalvia). The Biological Index (BI) was then calculated for each sample using the following formula:

BI = 0*%EG(I) + 1.5*%EG(II) + 3*%EG(III) + 4.5*%EG(IV) + 6*%EG(V)

Species richness (S) and Shannon-Weiner diversity index (H') were calculated for all species (including those that were not assigned an EG code (e.g., *Palposyllis prosostoma*, Gastropoda, and Bivalvia, etc.) using PRIMER. These four parameters (salinity code, BI, S, and H') were then run through the R script for the Northeast United States provided by M. Pelletier (personal communication 2024). The output number corresponded to benthic health condition within the following categories (Table 2): Bad (<0.20), Poor (0.20 to 0.39), Moderate (0.39 to 0.53), Good (0.53 to 0.77), and High (>0.77).

Table 2: US M-AMBI Benthic Health Conditions Categories and Scores

US M-AMBI Category	US M-AMBI Score
High	>0.77
Good	0.53-0.77
Moderate	0.39-0.53
Poor	0.20-0.39
Bad	<0.20

In addition to the US M-AMBI scores, AMBI scores and corresponding categories[2] (M. Pelletier personal communication 2024) were reported. AMBI is an abundance-weighted, tolerance value index that assesses habitat condition based upon the relative abundance of taxa in different tolerance value groups (i.e., EG codes) but does not account for salinity. While M-AMBI, in addition to factoring in the AMBI metric, also includes diversity, species richness, percentage of oligochaetes (for all marine/estuarine salinity categories), and salinity. The reporting of the two indices can be helpful to explain habitats for which the US M-AMBI scores do not appear to correlate with other information (e.g., community parameters, BBC Health Scores, high percentage of silt and/or TOC, etc.). US M-AMBI is reported in the National Coastal Condition Assessment as a condition indicator. AMBI is a metric used to calculate M-AMBI, and thus is not used solely as an indicator. However, both US M-AMBI scores/categories and the categories associated with AMBI can be considered together when trying to understand nuances of complex estuaries, such as the Onset and Buttermilk Bay System.

3 Results and Discussion

3.1 Water Quality

Water quality in the Onset and Buttermilk Bay System in 2023 was characterized by measuring four parameters at each of the 14 sampling locations: water temperature, DO, pH, and salinity (Appendix A). As mentioned above, the Onset and Buttermilk Bay System is designated as Class SA waters. The criteria for SA waters are that DO shall not be less than 6.0 mg/L, single temperature measurements shall not exceed 29.4°C (85°F) or a maximum daily mean of 80°F (26.7°C), and pH shall be between 6.5 and 8.5 standard units and not more than 0.2 standard units outside of the natural background range (MassDEP 2021). All water quality readings recorded during this survey met the Class SA water quality criteria.

3.2 Sediment Composition

Sediment conditions in the Onset and Buttermilk Bay System were characterized in 2023 by measuring two parameters at each sampling location where grab samples could be collected: (1) grain size and (2) total organic carbon (Table 3). In addition, the following field observations of the bottom conditions were recorded.

Sediments in the Onset and Buttermilk Bay System ranged from predominantly gravel at Station MEP-SE-031 (Cohasset Narrows) to sandy at Stations MEP-SE-036, 039, 034, 042, and 037 (Shell Point Bay,

[2] Benthic health categories associated with AMBI are as follows: <1.2 = undisturbed; 1.3-3.2 = slightly disturbed; 3.3-4.9 = moderately disturbed; 5.0-6.9 = heavily disturbed; 7= azoic.

Buttermilk Bay, and Little Buttermilk Bay, respectively) to silty sand in the remaining eight stations (Figure 3). Sediments at Station MEP-SE-032 in Broad Cove and Station MEP-SE-038 in Onset Bay had a sulfur odor. According to the field notes, there were several stations with algae clumps, *Crepidula* sp. shells (slipper shells), a few quahogs, polychaete worms, and crabs.

Notes on sediment and benthic infauna observed in the field at the following stations included:

- MEP-SE-039: Spider crab (*Libinia* sp.)
- MEP-SE-034: Polychaete worm (photo taken), live quahog (*Mercenaria mercenaria*) in jaw of grab, and quahog shell
- MEP-SE-042: Slipper shell (*Crepidula* sp.), very shallow so camera was not fully submerged
- MEP-SE-038: Very strong sulfur odor, seaweed (*Codium* sp.), and some shells
- MEP-SE-032: Slight sulfur odor, shell litter
- MEP-SE-043: Fine silty plume occurred when frame hit the bottom
- MEP-SE-040: Rockweed and *Codium* sp., seaweed, shells
- MEP-SE-036: Shell (photo taken)
- MEP-SE-035: Quahog seen in failed grab, small fish swam by
- MEP-SE-041: Polychaete worm, seaweed, algae
- MEP-SE-044: *Codium* sp., *Crepidula* sp. shells, algae, green crab
- MEP-SE-031: A lot of rocks covered with algae
- MEP-SE-033: Video was redone because the whole frame was not in the video on the first take.

No eelgrass was observed by the field crew at any of the stations.

3.2.1 Grain Size Analysis

Surface sediments collected at 14 sampling locations contained a range of gravel, sand, and silt summarized in Table 3 and Figure 3 below. The percentage of sediment types in the Onset and Buttermilk Bay System samples varied within and among basins. Percent silt in the Onset Bay embayments ranged from 5% at Station MEP-SE-040 in inner Onset Bay to 70% at Station MEP-SE-043 in mid-Onset Bay. Within the Buttermilk Bay embayments, percent silt ranged from 1% at Station MEP-SE-031 in inner Cohasset Narrows to 29% at Station MEP-SE-044 located in outer Cohasset Narrows. Interestingly, several stations in the innermost reaches of the Onset and Buttermilk Bay System that might be expected to have relatively high percentage of silt due to restricted tidal flushing, did not. For example, Station MEP-SE-033 in Muddy Cove (6% silt), 040 in inner Onset Bay (5% silt), and 034 and 039 in Buttermilk Bay (6% and 5% silt, respectively; Figure 3). In general, high percentages of organic matter deposition (e.g., silt) in sediment result in a relatively lower benthic habitat quality (Howes et al. 2014). Silty sediments are generally inhabited by low-diversity, shallow-dwelling organisms compared to high-diversity deep-burrowing organisms found in more sandy sediments (Howes et al. 2014).

3.2.2 Total Organic Carbon

Total organic carbon (TOC) can be an important parameter in characterizing the health status of a site. Organic matter in sediments can form water-soluble and water-insoluble complexes with metal ions and hydrous oxides, interact with clay minerals and bind particles together, adsorb and desorb both natural and man-made organic compounds, and absorb and release nutrients (Schumacher 2002). Three basic forms of carbon may be present in sediments: elemental carbon (from charcoal, soot, graphite, and coal), inorganic carbon (from geologic or soil parent material sources), and organic carbon (derived from the decomposition of plants and animals). In addition to the naturally occurring organic carbon sources, anthropogenic activities can also increase the total carbon content to sediment. For example, spills or releases of contaminants into the environment increase the total carbon content in the sediment. In general, though, the total carbon contribution from contaminants to the TOC content in sediment is relatively small to negligible unless a fresh spill has occurred (Schumacher 2002). The level of TOC can be used as a general indicator for sediment quality and impairment from organic waste and other anthropogenic pollutants (Hyland et al. 2005 and Pelletier et al. 2011). For example, sediments with percentages of TOC <1% are generally considered to be minimally impaired, between 1% and 3.5% moderately impaired, and >3.5% degraded (Hyland et al. 2005).

TOC in the stations sampled in the Onset and Buttermilk Bay System were relatively low, with all but two values below 1% (Table 3; Figure 4). Among embayments, TOC was variable ranging from 0.3% (Station MEP-SE-042, 034, and 039) in Buttermilk Bay to 3.7% (Station MEP-SE-037) in Little Buttermilk Bay. Within the Onset Bay embayment TOC ranged from 0.3% (Station MEP-SE-035 and 041) in outer Onset Bay to 1.8% (Station MEP-SE-038) in mid Onset Bay (Table 3; Figure 4). The relatively high TOC levels Station MEP-SE-037 in the innermost Little Buttermilk Bay, as well as MEP-SE-038 in the outer Onset Bay near the densely populated Nanumett Beach at the mouth of Pleasant Harbor were likely due to reduced tidal flushing resulting in increased sediment deposition. (Figure 2).

In general, the TOC was correlated with the percentage of silt with three exceptions. Stations MEP-SE-032 and 043 had a high percent silt (64% and 70%, respectively) and relatively low TOC (0.7% and 0.4%, respectively). Station MEP-SE-037 had a moderate percent silt (29%) and a high TOC (3.7%; Figure 4). The relatively high TOC at Station MEP-SE-037 is not surprising given the MassDEP Category 5[3] designation due to “Nutrient/Eutrophication and Biological Indicators” for Assessment Unit MA 95-76 (MassDEP 2023b).

[3] Waters requiring a TMDL (MassDEP 2023b).

Table 3. Results for the Onset and Buttermilk Bay System sediment grain size and TOC in 2023.

	Onset Bay System								Buttermilk Bay System					
	SE-036	**SE-033**	**SE-032**	**SE-040**	**SE-043**	**SE-038**	**SE-035**	**SE-041**	**SE-037**	**SE-042**	**SE-034**	**SE-039**	**SE-031**	**SE-044**
Gravel	0.35	1.05	0.46	0.00	3.90	0.00	0.34	17.09	9.83	5.73	0.51	5.66	89.21	18.30
Coarse Sand	1.22	1.80	2.22	0.03	4.45	0.13	0.46	3.44	27.61	15.67	11.70	12.00	6.02	7.51
Medium Sand	59.66	33.34	25.15	37.61	7.67	17.42	38.72	19.77	28.39	47.52	61.45	62.82	2.89	4.05
Very Fine Sand	28.51	57.65	8.46	57.65	13.80	15.00	52.52	45.50	4.85	6.39	20.46	14.78	0.83	41.14
Silt	10.25	6.16	63.71	4.72	70.19	67.45	7.96	14.21	29.32	24.69	5.88	4.74	1.04	29.01
TOC	0.69	0.52	0.66	0.41	0.38	1.81	0.28	0.31	3.73	0.27	0.33	0.32	0.85	0.93

Stations are organized from innermost (left) to outermost (right) within the Onset and Buttermilk Bay System; basins are separated by solid black lines.

a.

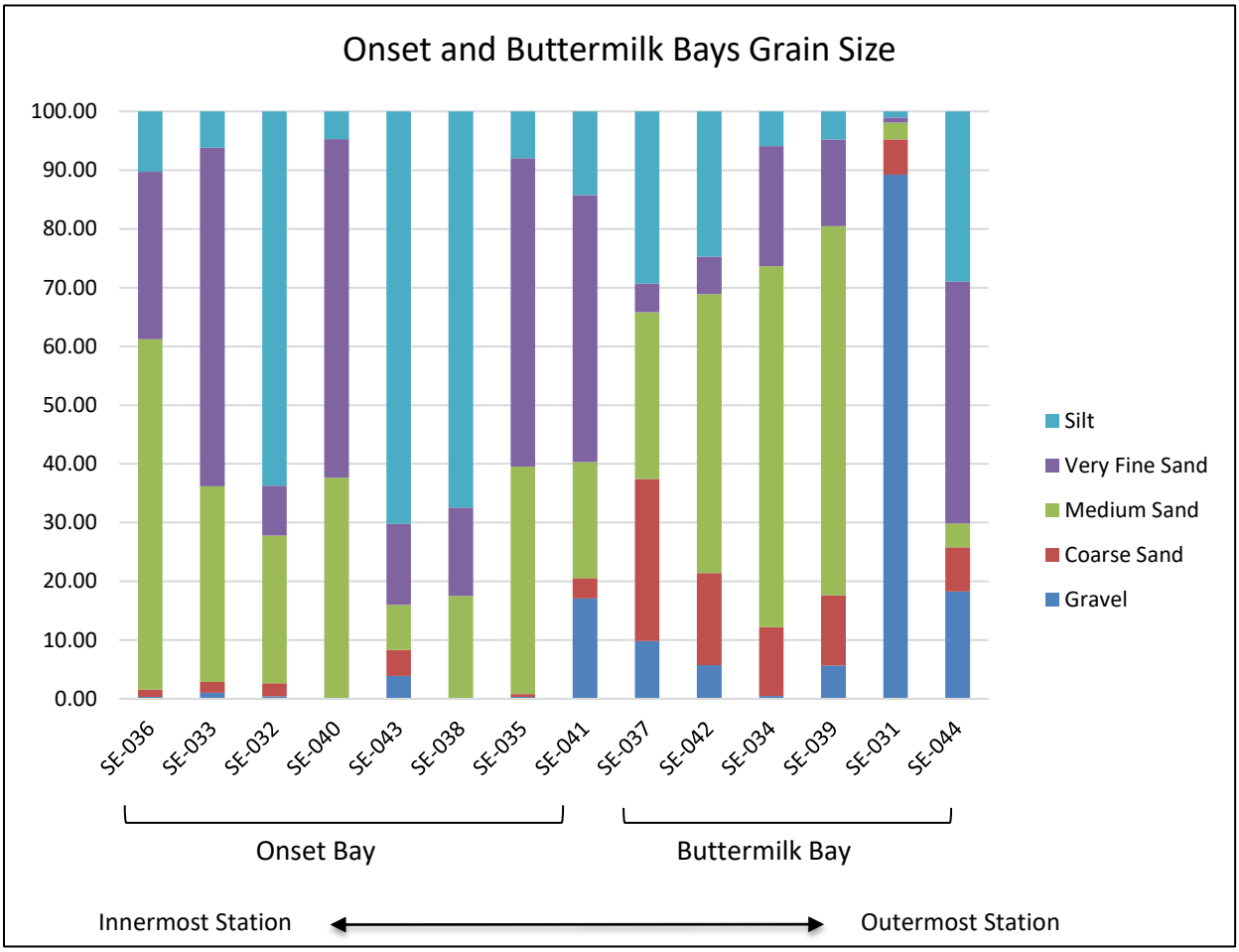


Figure 3. Onset and Buttermilk Bay System grain size analysis shown as a) a stacked bar graph and b) a ternary plot, fall 2023. In the ternary plot, Onset Bay stations are represented in blue, while Buttermilk Bay stations are in green. Darker shades indicate innermost stations, lighter shades indicate outermost stations.

b.

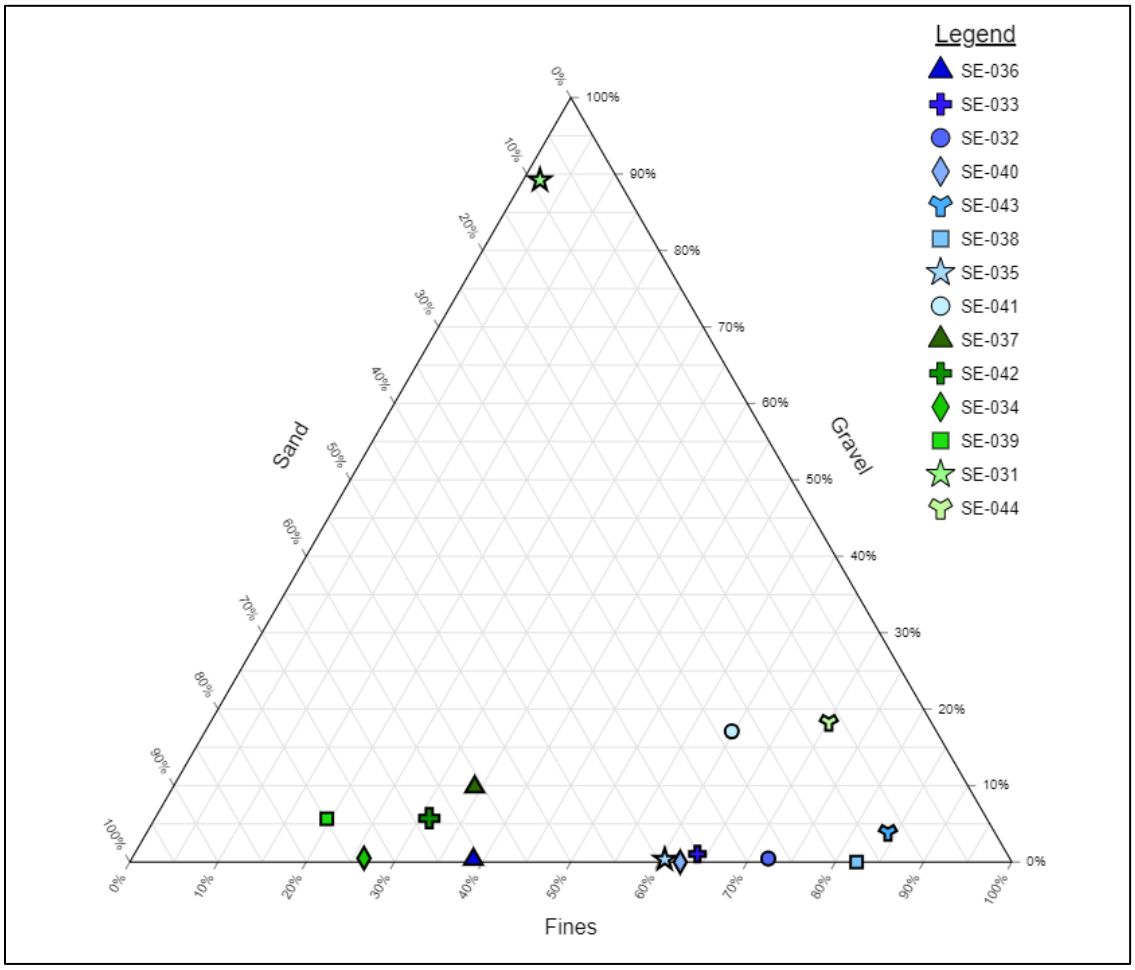


Figure 3. Continued. The data are displayed on this ternary plot so that stations with higher gravel proportions are plotted towards the top of the triangle, stations with higher sand proportions are plotted near the bottom left of the triangle, and stations with higher fines are plotted towards the bottom right of the triangle. The locations of the data points indicate the relative proportions of the three components of the soil. For example, station SE-031 was composed of approximately 89% gravel, 9% sand, and 2% fines.

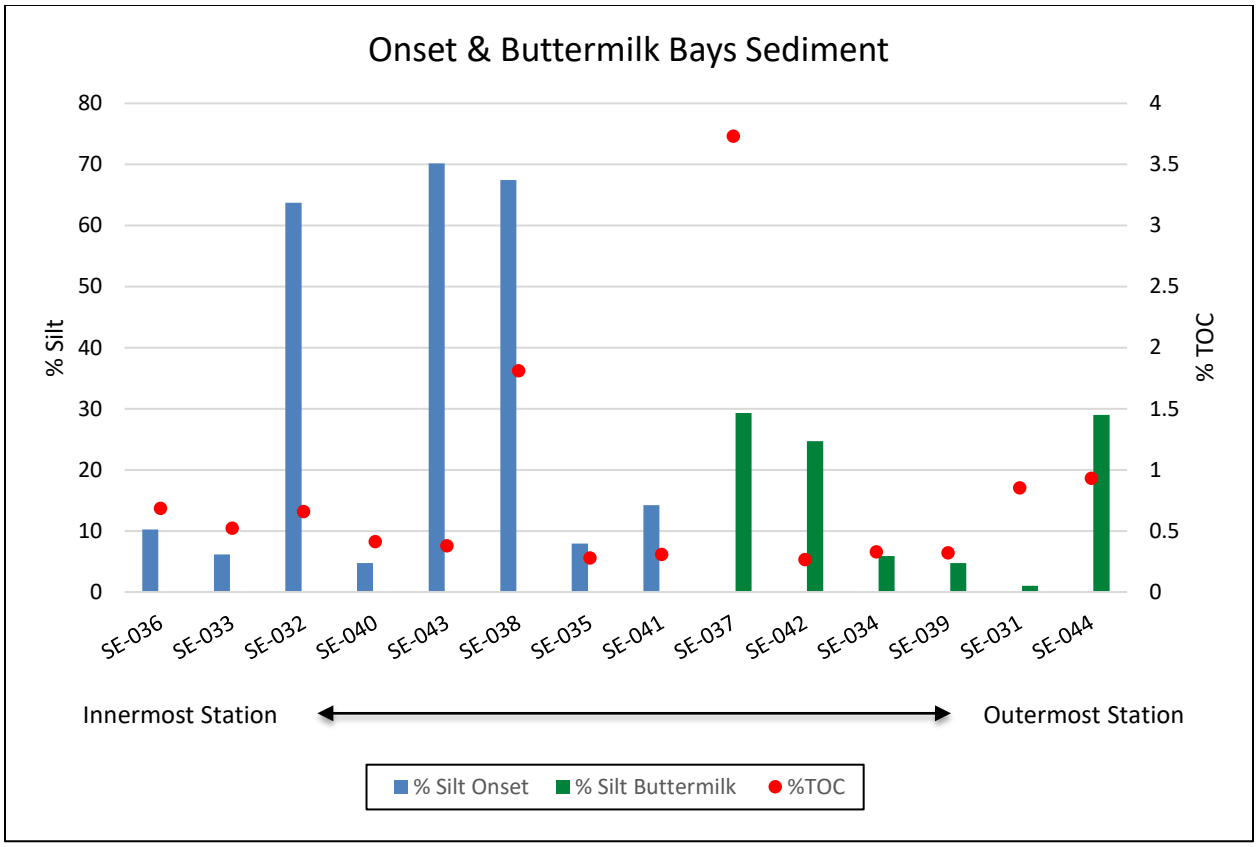
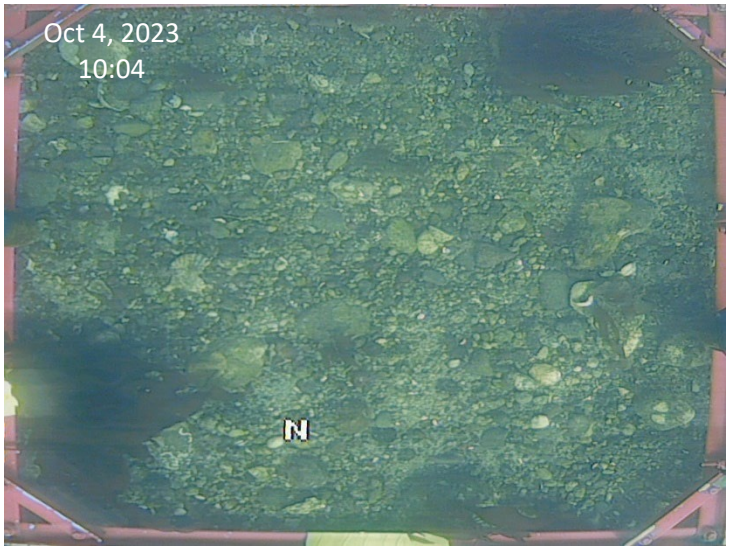


Figure 4. Onset and Buttermilk Bay System sediment: percent silt and TOC, 2023.

3.3 Underwater Digital Images

Digital photographs and videos were taken at each station in the Onset and Buttermilk Bay System. Still photographs for each sub-embayment can be found in Appendix B. Images of four representative habitat types recorded including coarse sand and gravel, silty sand with sparse vegetation, sandy with macroalgae and shells, and silty sand with seaweed are provided below in Figure 5. Eelgrass was not observed at any station except in sparse patches at Station MEP-SE-041, the outermost station in Onset Bay (Figure Appendix B-1i). This is consistent with results from the MassDEP Eelgrass Mapping Project that indicate eelgrass has declined in most areas of the Onset and Buttermilk Bay System from 1995 to 2017, the most recent year for which data are available (Costello & Kenworthy 2011, MassDEP 2018, WHG 2021).

a.



b.

c.

d.

Figure 5. Images of the Onset and Buttermilk Bay System bottom habitat: a) Station MEP-SE-031 (coarse sand, gravel), b) Station MEP-SE-034 (silty sand, sparse vegetation), c) Station MEP-SE-040 (sandy, macroalgae and shells), d) Station MEP-SE-044 (silty sand, seaweed).

3.4 Benthic Infauna Community

The 2023 Onset and Buttermilk Bay System benthic samples contained a total of 187 taxa, representing eight phyla (Table 4). The Onset and Buttermilk Bay benthic communities were characterized based on the following macroinvertebrate metrics: number of species (S), abundance (N), species richness (Magalef, D_{mg}), diversity (Shannon-Weiner, [H'] and Simpson's index [1-λ]), and evenness (Pielou, J'). In addition, Average Taxonomic Distinctness (ATD), cluster and non-metric multidimensional scaling (MDS) analyses, and US M-AMBI are presented to assess spatial and temporal trends in community composition within and between sub-embayments, and eventually between estuaries. Due to the complexity and size of the Onset and Buttermilk Bay System, the cluster and MDS analyses are presented first to provide groups based on similarity for which the remaining metrics could be discussed in the report. Since US M-AMBI incorporates several of the above metrics (i.e. species number, Shannon-Weiner diversity H', salinity category, and BI score [see Methods section above]), US M-AMBI was used as an overall summary of the benthic habitat health status.

3.4.1 Dominant taxonomic groups and species

Among all stations, a total of 36,949 individuals from 187 taxa were identified in the 2023 Onset and Buttermilk Bay System benthos (Table 4). These taxa represented eight phyla: Annelida (aquatic earth worms and bristle worms), Arthropoda (amphipods, shrimp, crabs, and insects), Mollusca (bivalves and snails), Cnidaria (sea anemones), Chordata (tunicates), Echinodermata (sea cucumbers), Platyhelminthes (flat worms), and Nemertea (ribbon worms). A majority of the taxa (71%) were annelids (54% polychaetes and 16% oligochaetes), followed by arthropods (16%) and molluscs (12%). The remaining five phyla contributed a total of 0.9% to the abundance. The top ten taxa contributed 68% to the total abundance. The three most abundant taxa were oligochaetes (3,014 average number individuals per station, 16.3% of the total), bivalve *Gemma gemma* (1,800 individuals, 9.7% of the total), and polychaete *Mediomastus ambiseta* (1,491 individuals, 8.1%).

Table 4. Taxonomic list for Onset and Buttermilk Bay benthos, 2023.

Phylum	Class/Order	Taxon
Annelida	**Polychaeta**	*Alitta succinea*
		Alitta virens
		Arabella iricolor
		Aricidea (Acmira) catherinae
		Brania wellfleetensis
		Capitella capitata complex
		Capitella teleta
		Clymenella torquata
		Ctenodrilus serratus
		Diopatra cuprea
		Dipolydora giardi
		Dipolydora quadrilobata
		Dipolydora socialis
		Dipolydora sp. A
		Dodecaceria sp.
		Drilonereis longa
		Enoplobranchus sanguineus
		Erinaceusyllis erinaceus
		Eteone longa
		Euclymene collaris
		Eumida sanguinea
		Exogone dispar
		Exogone naidina
		Fabricia stellaris
		Glycera americana
		Glycinde multidens
		Heteromastus filiformis
		Hypereteone heteropoda
		Hypereteone lactea
		Leitoscoloplos robustus
		Lysidice unicornis
		Marenzelleria sp.
		Marphysa sanguinea
		Marycarmenia gaspeensis
		Mediomastus ambiseta
		Mediomastus californiensis
		Melinna maculata
		Micromaldane ornithochaeta
		Micronephthys neotena
		Microphthalmus aberrans
		Microphthalmus sczelkowii
		Neanthes arenaceodentata
		Neoamphitrite figulus
		Nephtys picta
		Nicolea zostericola
		Ninoe nigripes
		Notomastus latericeus
		Notomastus sp. A Ewing
		Odontosyllis fulgurans
		Opisthodonta longocirrata
		Oxydromus obscurus
		Palposyllis prosostoma
		Paranaitis speciosa
		Parasabella microphthalma
		Parougia caeca
		Pectinaria gouldii
		Phascolopsis gouldii
		Phyllodoce arenae
		Pista mediterranea
		Pista sp.
		Platynereis dumerilii
		Polycirrus eximius
		Polydora aggregata
		Polydora cornuta
		Polygordius jouinae
		Prionospio heterobranchia
		Pygospio elegans
		Sabaco elongatus
		Salvatoria clavata
		Scolelepis (Parascolelepis) texana
		Scoletoma tenuis
		Sphaerosyllis perkinsi
		Sphaerosyllis taylori
		Spio c.f. filicornis
		Spiochaetopterus oculatus
		Spiophanes bombyx
		Streblospio benedicti
		Streptosyllis verrilli
		Streptosyllis websteri
		Syllides floridanus
		Syllis gracilis
		Terebella lapidaria
		Tharyx acutus
		Tharyx sp. A
	Oligochaeta	Oligochaeta
Arthropoda	**Amphipoda**	*Ampelisca abdita*
		Ampelisca vadorum
		Ampelisca verrilli
		Ampithoe longimana
		Ampithoe valida
		Apocorophium acutum
		Batea catharinensis
		Caprella penantis
		Crassicorophium bonellii
		Cymadusa compta
		Deutella incerta
		Elasmopus levis
		Eobrolgus spinosus

Table 4. Continued.

Phylum	Class	Taxon	Phylum	Class	Taxon
Arthropoda	**Amphipoda**	*Ericthonius brasiliensis*	**Arthropoda**	**Tanaidacea**	Leptocheliidae
		Gammaropsis maculata	**Mollusca**	**Gastropoda**	*Crepidula convexa*
		Grandidierella japonica			*Crepidula fornicata*
		Idunella barnardi			*Crepidula* sp.
		Idunella clymenellae			*Eupleura caudata*
		Jassa marmorata			*Fargoa gibbosa*
		Leptocheirus pinguis			*Haminella solitaria*
		Lysianopsis alba			*Ilyanassa trivittata*
		Microdeutopus anomalus			*Japonactaeon punctostriatus*
		Microdeutopus gryllotalpa			*Lacuna vincta*
		Microprotopus raneyi			*Margarites helicinus*
		Monocorophium acherusicum			*Odostomia eburnea*
		Monocorophium insidiosum			*Onchidoris sp.*
		Paracaprella tenuis			*Turbonilla interrupta*
		Phoxocephalus holbolli			Turbonillinae
		Rudilemboides naglei	**Cnidaria**	**Actiniaria**	*Edwardsia elegans*
	Cumacea	*Cyclaspis varians*			*Haloclava producta*
		Leucon americanus			*Nematostella vectensis*
		Oxyurostylis smithi	**Chordata**	**Ascidiacia**	*Molgula* sp.
	Decapoda	*Carcinus maenas*	**Echinodermata**	**Holothuroidea**	Apodida
		Dyspanopeus sayi	**Nemertea**	**Hoplonemertea**	*Amphiporus ochraceus*
		Hippolyte zostericola		**Hoplonemertea**	*Amphiporus* sp.
		Pagurus annulipes		**Hoplonemertea**	*Correanemertes bioculatus*
		Pagurus longicarpus		**Hoplonemertea**	*Tetrastemma candidum*
		Tubicolixa chaetopterana		**Hoplonemertea**	*Tetrastemma* sp.
		Crangon septemspinosa		**Hoplonemertea**	*Zygonemertes virescens*
	Isopoda	*Edotia triloba*		**Palaeonemertea**	*Tubulanus* sp.
		Erichsonella filiformis	**Platyhelminthes**		distinct type "17 NAI"
		Ianiropsis serricaudis	**Platyhelminthes**		distinct type "22 NAI"
	Pycnogonida	*Anoplodactylus petiolatus*	**Platyhelminthes**		distinct type "23 NAI"
		Callipallene brevirostris	**Platyhelminthes**		distinct type "5 NAI"
Mollusca	**Bivalvia**	*Ameritella agilis*			
		Ennucula delphinodonta			
		Gemma gemma			
		Kurtiella planulata			
		Laevicardium mortoni			
		Lyonsia hyalina			
		Macoploma tenta			
		Mercenaria mercenaria			
		Mytilidae			
		Nucula proxima			
		Pectinidae			
		Solemya velum			
		Spisula solidissima			
		Tagelus divisus			
	Gastropoda	*Acteocina canaliculata*			
		Astyris lunata			
		Bittiolum alternatum			
		Boonea seminuda			
		Caecum pulchellum			
		Coryphella sp.			
		Cotonopsis lafresnayi			

3.4.2 Cluster and non-metric multidimensional scaling (MDS)

Onset and Buttermilk Bay System is a large, widespread embayment comprising seven sub-embayments: Onset Bay, Shell Point Bay, Muddy Cove, Broad Cove, Cohasset Narrows, Buttermilk Bay, and Little Buttermilk Bay. As indicated above, benthic infaunal grabs were collected at 14 stations in the sub-embayments or basins. Multivariate analyses were used to assess spatial patterns in the infaunal assemblages at the Onset and Buttermilk Bay sampling stations. The cluster analysis identified two group assemblages and three single-station groupings summarized in Table 5 and Figure 6:

- Group 1 -Outer Bay (Stations MEP-SE-035 and 041 in outer Onset Bay, and MEP-SE-044 in outer Cohasset Narrows);
- Group 2 – Inner Bay (Station MEP-SE-032 in Broad Cove, 033 in Muddy Cove, 040 in inner Onset Bay, 036 in Shell Point Bay, 043 in mid Onset Bay, 042, 034, and 039, in inner Buttermilk Bay, and 037 in inner Little Buttermilk Bay;
- Group 3 – Mid-Bay (Station MEP-SE-038 in mid-Onset Bay);
- Group 4 – Little Buttermilk Bay (Station MEP-SE-037); and
- Group 5 – Inner Cohasset Narrows (Station MEP-SE-031).

The patterns identified through cluster analysis were confirmed in the MDS ordination plot (Figure 7). The similarity among groups was 47% for Group 1 and 46% for Group 2 (dark blue dashed lines in Figure 7). The single-group stations MEP-SE-031 and MEP-SE-037 were similar (i.e., in relative proximity) to Group 1 (36% similarity) and Group 2 (37% similarity), respectively (Figure 7). Although the MDS detected enough of a difference to separate Stations MEP-SE-031 and MEP-SE-037, they have been included in Group 1 and Group2, respectively for the following sections and analyses in this report due to the similarity: MEP-SE-031 has 36% similarity with Group 1 and MEP-SE-037 Group 2 has 37% similarity with Group 2 (Figure 6). Station MEP-SE-038 (Group 3) was an outlier, with only 20% similarity to all other stations, and thus was not included in either group, and was analyzed separately.

Table 5. Summary of groupings identified in the cluster and MDS plots of the Onset and Buttermilk Bay System benthic infauna, 2023.

Group Number	Group Name	Station MEP-SE	Subembayment in Onset & Buttermilk Bay System
1	Outer Bay	35	Outer Onset Bay
		41	Outer Onset Bay
		44	Outer Cohasset Narrows
2	Inner Bay	32	Broad Cove
		33	Muddy Cove
		40	Inner Onset Bay
		36	Shell Point Bay
		43	Mid Onset Bay
		42	Inner Buttermilk Bay
		34	Inner Buttermilk Bay
		39	Inner Buttermilk Bay
3	Middle Bay	38	Mid Onset Bay
4*	Little Buttermilk Bay	37*	Inner Little Buttermilk Bay
5*	Inner Cohasset Narrows	31*	Inner Cohasset Narrows

*Based on similarity levels, Station MEP-SE-31 has been added to Group 1 and Station MEP-SE-037 has been added to Group 2 for analysis in the following sections of this report.

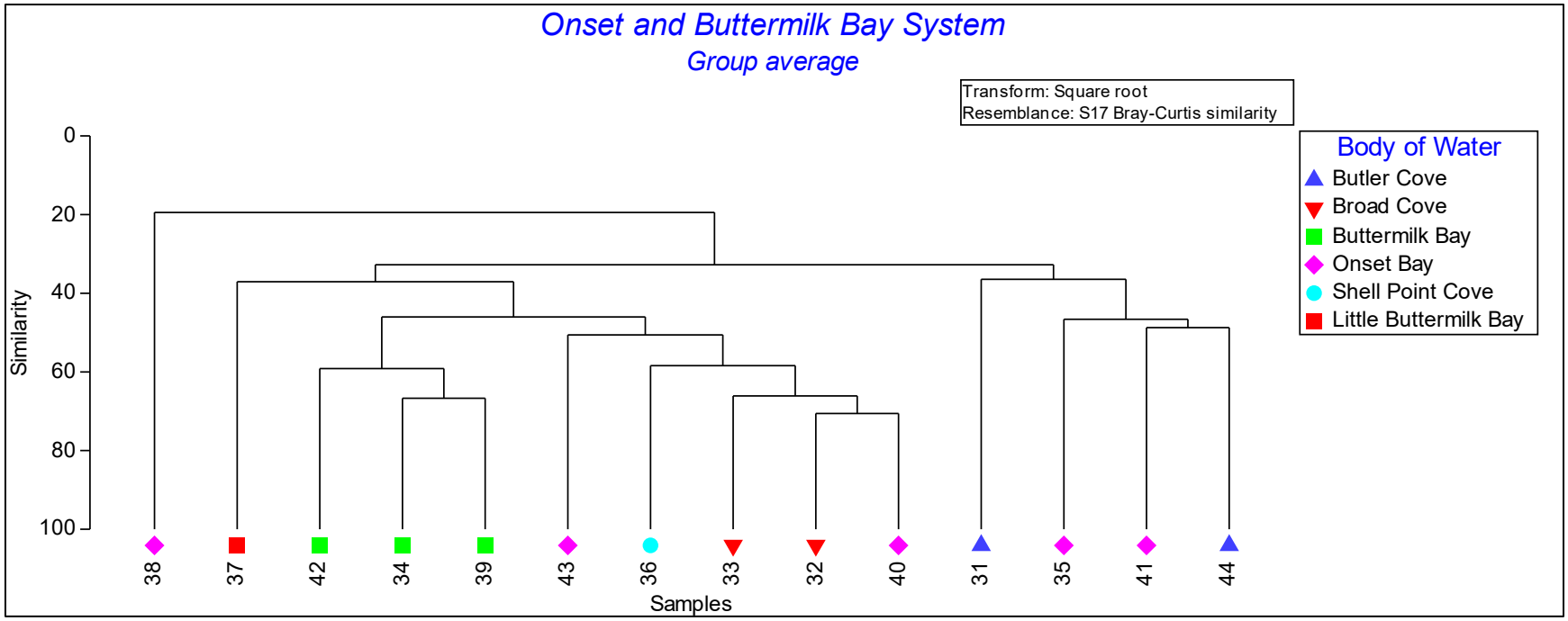


Figure 6. Cluster analysis results of the 2023 Onset and Buttermilk Bay System infauna (Station MEP-SE-0XX).

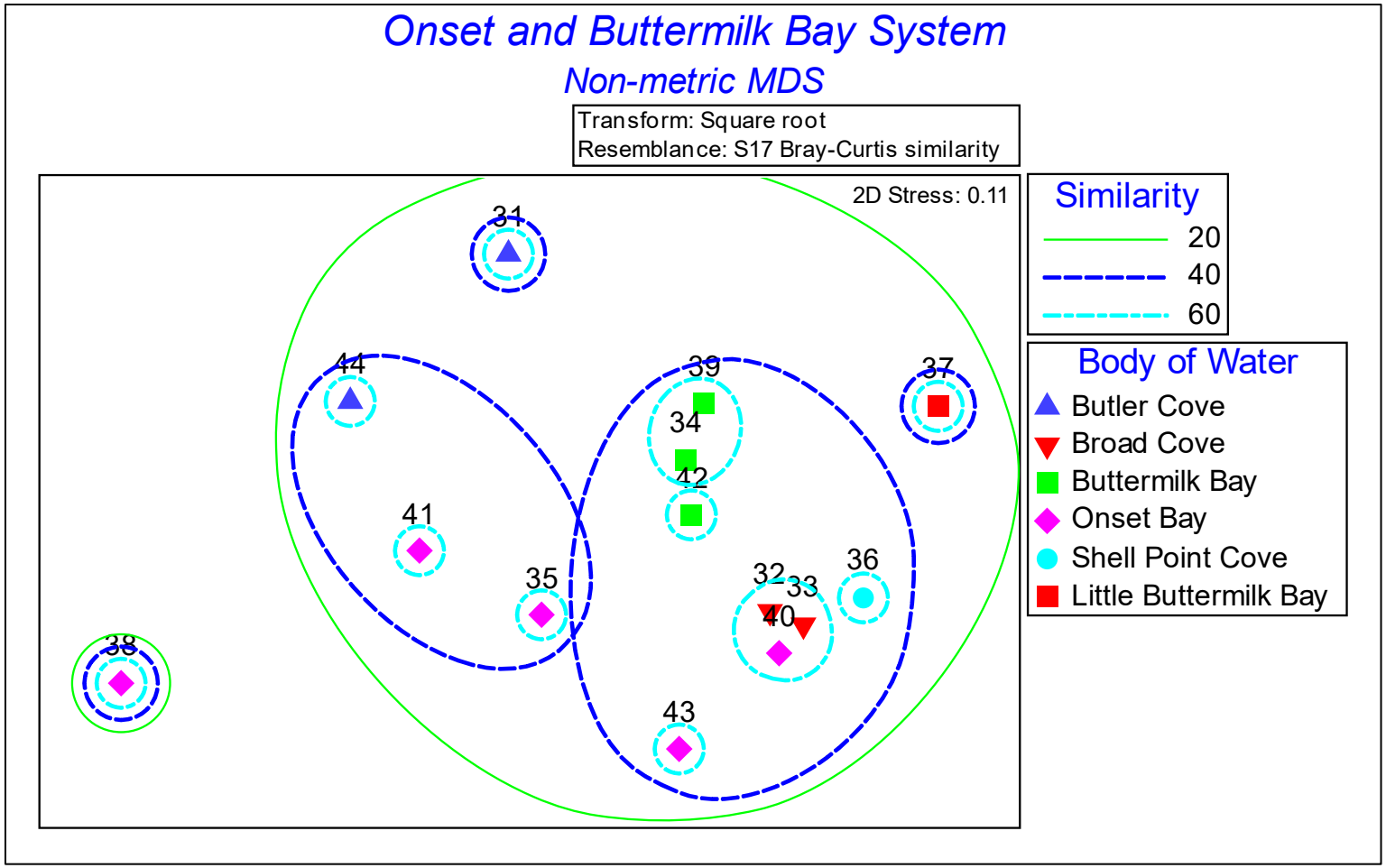


Figure 7. MDS ordination plot of Onset and Buttermilk Bay 2023 infaunal benthic samples. Each point on the plot represents one of the 14 stations (MEP-SE-0XX). The symbols represent the subembayments within the Onset and Buttermilk Bay System.

3.4.3 Dominant taxonomic groups and species

The top three taxonomic groups for each of the three groupings are presented in Table 6 and Figure 8. Polychaetes were the most abundant taxonomic group in all three groupings, ranging from 42% in Group 1 (Outer Bay) to 78% in Group 3 (Mid-Bay [single-station group]). Oligochaetes were the second most abundant taxon in Group 1 and 2 but contributed just 1% to the total in Group 3. Amphipoda, including gammarid (laterally compressed shrimp-like animals) and caprellid (skeleton shrimp) amphipods, were the third most abundant taxonomic group in Group 1 and 2 and second most abundant in Group 3. Percent contribution of amphipods ranged from 12% in Group 3 to 18% in Group 1.

Table 6. Percent contribution of taxonomic groups in the Outer Bay (Group 1), Inner Bay (Group 2), and Mid-Bay (Group 3), Onset and Buttermilk Bay System benthos, 2023.

Taxonomic Group/Order	Group 1* (4 stations)	Group 2 (9 stations)	Group 3 (1 station)
Polychaeta	41.9	56.8	78.3
Oligochaeta	24.8	15.0	1.3
Amphipoda	17.9	13.2	11.5
Gastropoda	7.5		5.2
Bivalvia		12.0	1.3
Isopoda	3.4	0.8	
Tanaidacea			1.7

*Groups were based on MDS and Cluster results (See Section 3.4.2 and Table 5 for grouping details).

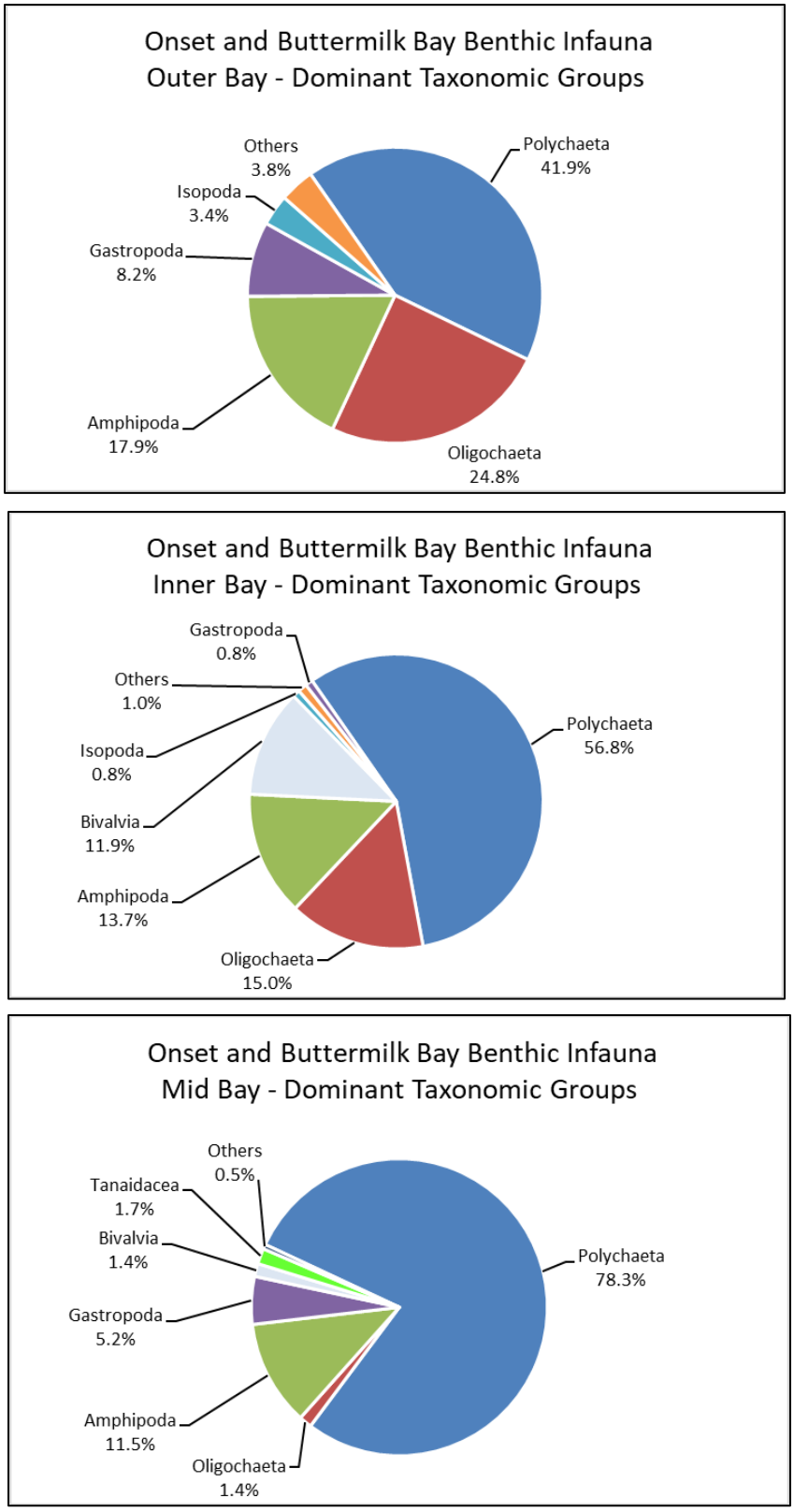


Figure 8. Percentage of benthic groups in the Onset and Buttermilk Bay System: Outer Bay (Group 1), Inner Bay (Group 2), and Mid-Bay (Group 3), benthos, 2023.

The top five dominant species differed between the Outer Bay, Inner Bay, and mid-Bay groupings in the Onset and Buttermilk Bay System with almost no overlap (Table 7, Figure 9). The exception was oligochaetes, which were the most abundant taxon in Group 1 and Group 2, contributing 25% and 15%, respectively. Polychaete *Capitella capitata* complex was the most abundant species in Group 3 and the second most abundant in Group 1, contributing 73% and 10%, respectively. *C. capitata* is a complex of cryptic species in the family Capitellidae, which are among the most abundant polychaetes in soft-bottom communities (Blake and Ruff 2007).

The remaining top contributors were different in each group. The third dominant species in Group 1 was polychaete *Salvatoria clavata* (8%), followed by polychaete *Polydora cornuta* (3%), and Isopod (dorso-ventrally compressed crustaceans) *Ianiropsis serricaudis* (3%). *S. clavata*, is a member of the Syllidae family, which are usually associated with undisturbed habitats (Musco 2012). However, there are also other examples of habitats in which syllids are considered opportunistic species, where their abundance increases with elevated environmental stressors (Romero et al. 2019). In addition, small-sized polychaetes (like syllids) have been categorized as a second-order opportunistic species, since their presence and abundance increase in disturbed conditions (Borja et al. 2000). In a study of benthic assemblages in marinas in the Iberian Peninsula, *S. clavata* was by far the most dominant species in the study area (40% of total abundance) and was present in 22 out of the 42 marinas sampled (Romero et al. 2019). Generalized Linear Models indicated that the highest predicted abundance of *S. clavata* occurred at marinas where concentration of nutrients and heavy metals were highest (Romero et al. 2019). The most significant factor associated with high abundance of *S. clavata* was nutrient enrichment and elevated concentrations of nitrogen and phosphorus in the water column (Romero et al. 2019). In addition, several species, including *S. clavata*, were associated with marinas in which higher concentrations of nitrogen, zinc, and copper were recorded (Romero et al. 2019). Thus, although *S. clavata* is not currently considered a pollution tolerant or sensitive species (Pelletier et al. 2010), such studies indicate that it may be a useful indicator of pollutants associated with marinas.

Top contributors in Group 2 included bivalve *Gemma gemma*, ranking second highest species (12%), polychaete *Mediomastus ambiseta* (9%), and polychaetes *Streblospio benedicti* and *Scoletoma tenuis* (7%). *M. ambiseta* is a pollution-tolerant species known to occur in shallow coastal waters in mud and muddy sand sediments. *M. ambiseta* lives in vertical mucus tubes and has been recorded in association with *S. benedicti,* as was the case in the Inner Onset Bay and Buttermilk Bay. *S. benedicti* occurs in mudflats and soft sediments of estuaries and coastal waters and tolerates a broad range of temperatures and salinities. *S. benedicti* is tolerant to high organic content and pollution, flourishing in disturbed environments. It is considered an opportunistic pioneering species (Zakas and Wares 2012; Kocheshkova and Matviy 2009; Detwiler et al. 2002).

In addition to *C. capitata* complex, dominant species in Group 3 consisted of gammarid amphipod *Cymadusa compta* (5%), gastropod *Astyris lunata* (5%), amphipod *Microdeutopus gryllotalpa* (4%), and tanaid shrimp Leptochelidae (2%).

Table 7. Percent contribution of the top five benthic species in Onset and Buttermilk Bay System: Outer Bay (Group 1), Inner Bay (Group 2), and Mid-Bay (Group 3), benthos, 2023.

Taxanomic Group	Taxa	Group		
		Outer Bay (%)	Inner Bay (%)	Mid Bay (%)
Oligochaeta	Oligochaeta	24.8	15.0	
Polychaeta	*Capitella capitata* complex	9.9		73.4
Bivalvia	*Gemma gemma*		11.5	
Polychaeta	*Salvatoria clavata*	7.6		
Polychaeta	*Polydora cornuta*	3.2		
Isopoda	*Ianiropsis serricaudis*	3.2		
Polychaeta	*Mediomastus ambiseta*		9.4	
Polychaeta	*Streblospio benedicti*		7.9	
Polychaeta	*Scoletoma tenuis*		6.9	
Amphipoda	*Cymadusa compta*			4.9
Gastropoda	*Astyris lunata*			4.5
Amphipoda	*Microdeutopus gryllotalpa*			4.2
Tanaid	Leptocheliidae			1.7
Others		51.3 (n = 123)	49.3 (n = 143)	11.2 (n = 16)

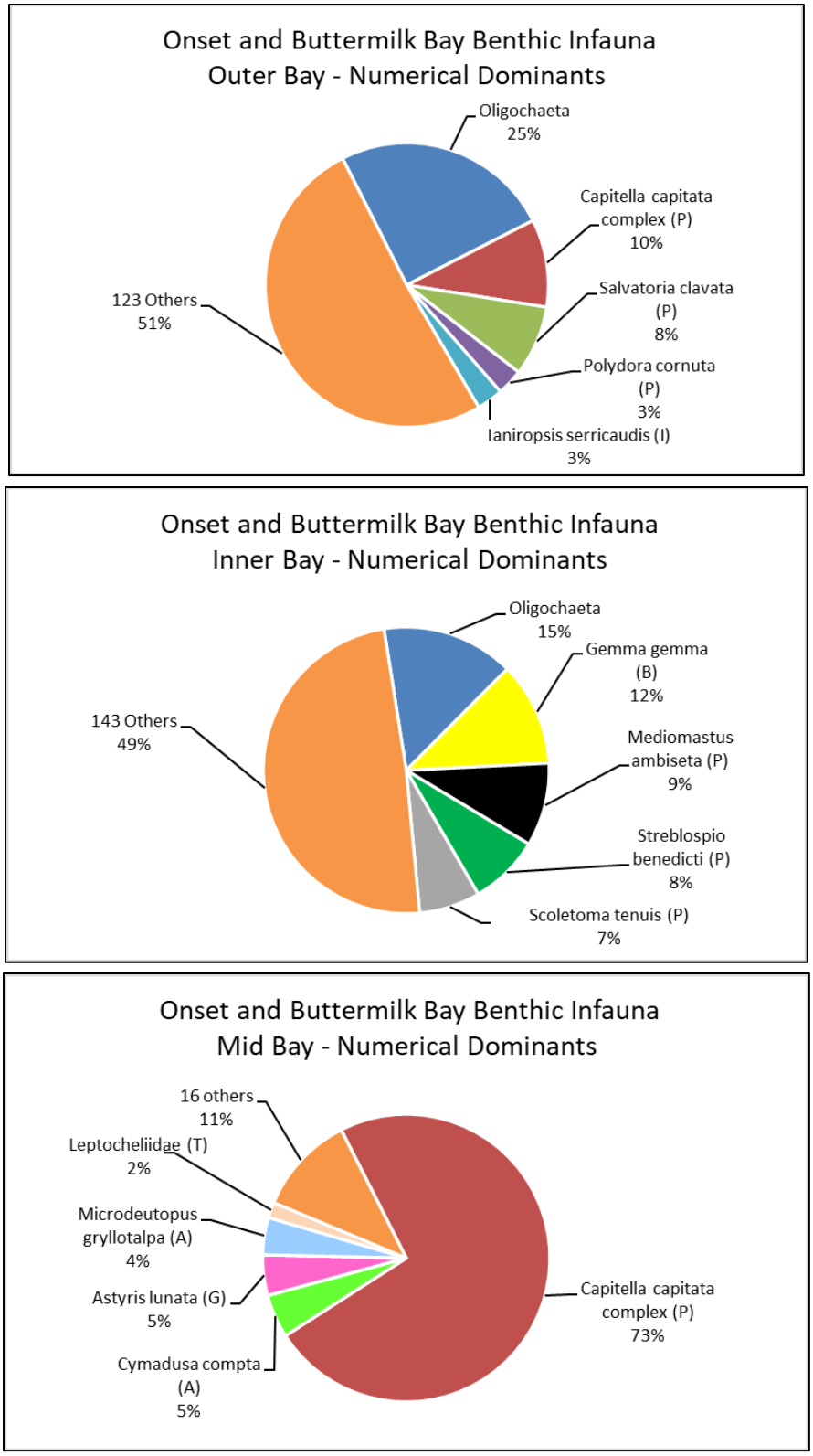


Figure 9. Onset and Buttermilk Bay System: Outer Bay (Group 1), Inner Bay (Group 2), and Mid-Bay (Group 3), benthos, 2023 P = Polychaete, B = Bivalve, I = Isopod, A = Amphipod, T = Tanaid, and G = Gastropod.

3.4.4 Diversity, richness, and evenness indices

When comparing the three groupings in terms of the number of species and abundance, the Inner and Outer Bays appear to have relatively higher quality benthic habitat compared to the Mid-Bay. The average number of taxa was higher in the Outer Bay (63 taxa, ranging from 50 to 70 taxa) compared to the Inner Bay (59 taxa, ranging from 43 to 77; Table 8; Figure 10). The number of taxa in the Mid-Bay (single-station group) was relatively low (21 taxa). The mean number of individuals was higher in the Inner Bay (1,744 individuals, ranging from 778 to 3,126 individuals per sample) compared to the Outer Bay (660 individuals, ranging from 406 to 943 individuals). The number of individuals in the Mid-Bay, 143 individuals, was much lower (Table 8, Figure 11).

Table 8. Onset and Buttermilk Bay infaunal community parameters by station, 2023.

Group Name	Group Number	Station	S	N	d	J'	H'(loge)	1-Lambda'	% Oligochaetes
Outer Bay	1	MEP_SE_031	70	723	10.48	0.78	3.31	0.94	8.2
		MEP_SE_035	50	568	7.73	0.60	2.35	0.82	35.3
		MEP_SE_041	65	406	10.66	0.71	2.95	0.91	20.4
		MEP_SE_044	64	943	9.20	0.61	2.54	0.84	33.1
Inner Bay	2	MEP_SE_032	58	1668	7.68	0.66	2.69	0.90	10.0
		MEP_SE_033	55	1033	7.78	0.63	2.52	0.88	1.4
		MEP_SE_034	77	3126	9.44	0.54	2.34	0.81	18.6
		MEP_SE_036	56	1468	7.54	0.65	2.60	0.86	9.7
		MEP_SE_037	43	1767	5.62	0.49	1.84	0.69	0.6
		MEP_SE_039	59	2977	7.25	0.61	2.50	0.83	38.2
		MEP_SE_040	56	1029	7.93	0.58	2.35	0.83	6.7
		MEP_SE_042	62	1848	8.11	0.70	2.87	0.92	5.7
		MEP_SE_043	60	778	8.86	0.59	2.41	0.85	17.0
Mid Bay	3	MEP_SE_038	21	143	4.03	0.41	1.26	0.46	1.4

S = Total number of distinct taxa in both replicates, N = mean number of individuals, d = Margalef's species richness, J' = Pielou's evenness, H' = Shannon-Weiner diversity index, and 1-λ = Simpson diversity[4].

[4] D, J', H', and 1-λ were calculated using station data.

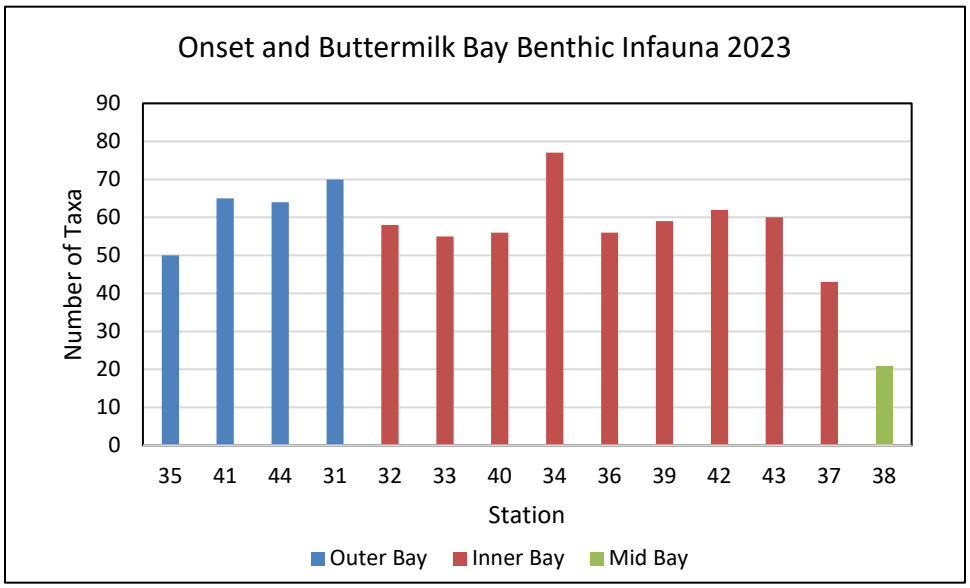


Figure 10. Total number of distinct taxa per station the Onset and Buttermilk Bay System Benthos, 2023: Outer Bay (blue bars), Inner Bay (red bars), and Mid Bay (green bar).

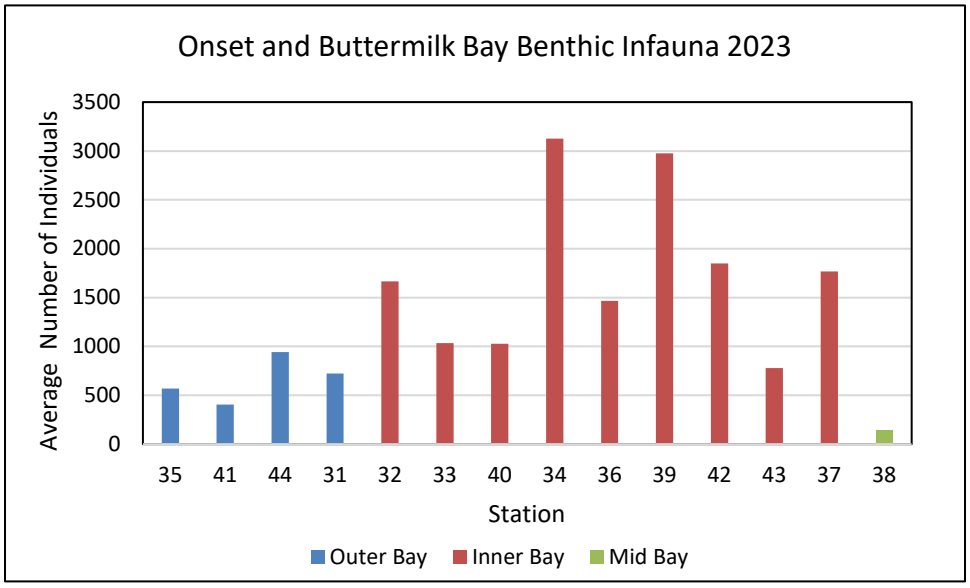


Figure 11. Mean number of individuals for the Onset and Buttermilk Bay System Benthos, 2023: Outer Bay (blue bars), Inner Bay (red bars), and Mid Bay (green bar).

Overall, diversity, richness, and evenness indices were similar between the Outer Bay and Inner Bay indicating relatively healthy habitats while the Mid-Bay indices indicated a relatively degraded habitat. The Shannon-Wiener diversity index is a function of the number of different taxa in a sample, the number of individuals per taxa, and the total number of individuals. H' increases with the number of species in the community and when a more even distribution of numbers among taxa is found. H' ranges

from 0 when only one species is present to 5.0 when many taxa are found in equal numbers of individuals. Evenness is another expression of how individuals are distributed among different species or taxa. Pielou's evenness index (J') ranges from 0 to 1 and is essentially the reverse of dominance and therefore, a sample with low evenness would be highly dominated by a small number of the taxa present.

The average Shannon Wiener diversity index (H') was higher in the Outer Bay (2.8 with a range of 2.4 to 3.3) compared to the Inner Bay (mean of 2.5, range of 1.8 to 2.9) and the Mid-Bay was much lower (1.3; Table 8; Figure 12). Similarly, Margalef's species richness (D_{mg}), and Simpson's diversity (1-λ) indices indicated that the Outer and Inner Bays had higher habitat quality (e.g., higher richness and diversity, and lower evenness) compared to the Mid-Bay station (Figure 13 and Figure 14, respectively). Pielou's evenness (J') was lowest in the Mid-Bay (0.4) compared to the Outer Bay (average 0.7, with a range of 0.6 to 0.8) and the Inner Bay (0.6 with a range of 0.5 to 0.7; Figure 15). Station MEP-SE-038 had the lowest values in each of the four community parameters (Figure 12 – 15). This is not surprising due to the low number of taxa (n = 21), very low number of individuals (n = 143) and numerical dominance (73%) of a single species (*C. capitella* complex). The proportion of silt (68%) in the sediment at Station MEP-SE-038 was very high and TOC was the second highest in the survey (1.8%) suggesting a relatively low current, possibly due to an eddy-effect from Onset Island which is approximately 250 m southeast of the Station location. However, more data are needed to clarify the ecological and bathymetric conditions in this area.

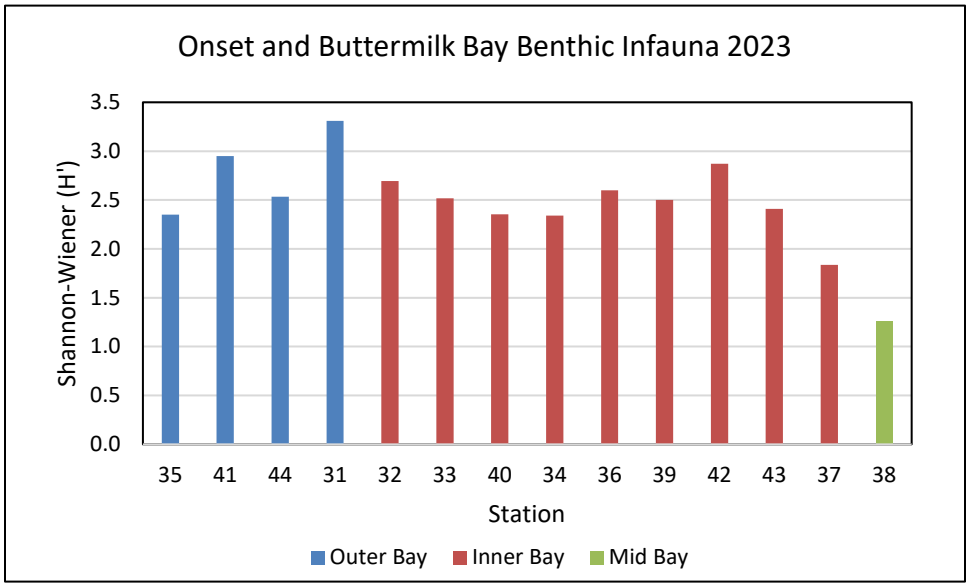


Figure 12. Shannon-Weiner diversity indices for the Onset and Buttermilk Bay System Benthos, 2023: Outer Bay (blue bars), Inner Bay (red bars), and Mid Bay (green bar).

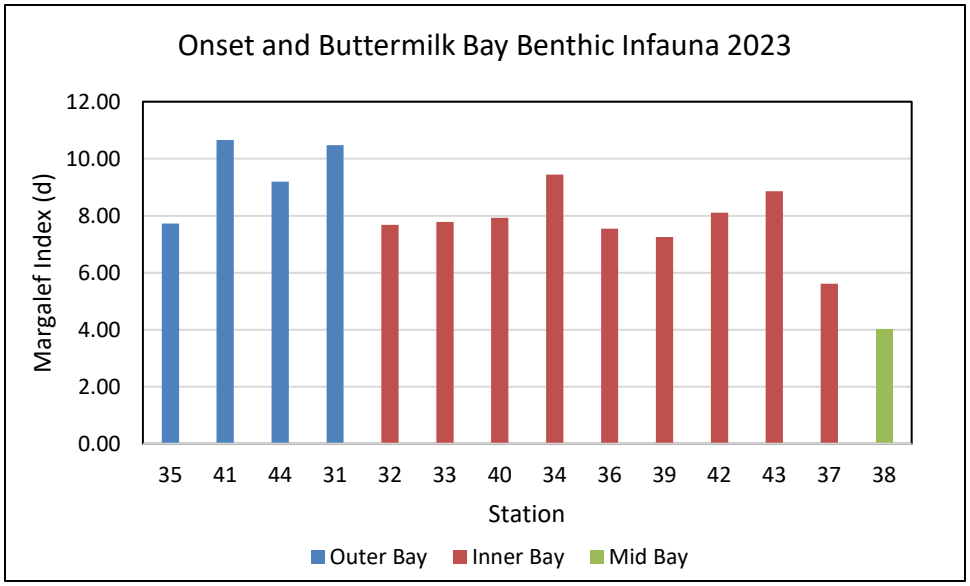


Figure 13. Margalef's species richness (d) indices for the Onset and Buttermilk Bay System Benthos, 2023: Outer Bay (blue bars), Inner Bay (red bars), and Mid Bay (green bar).

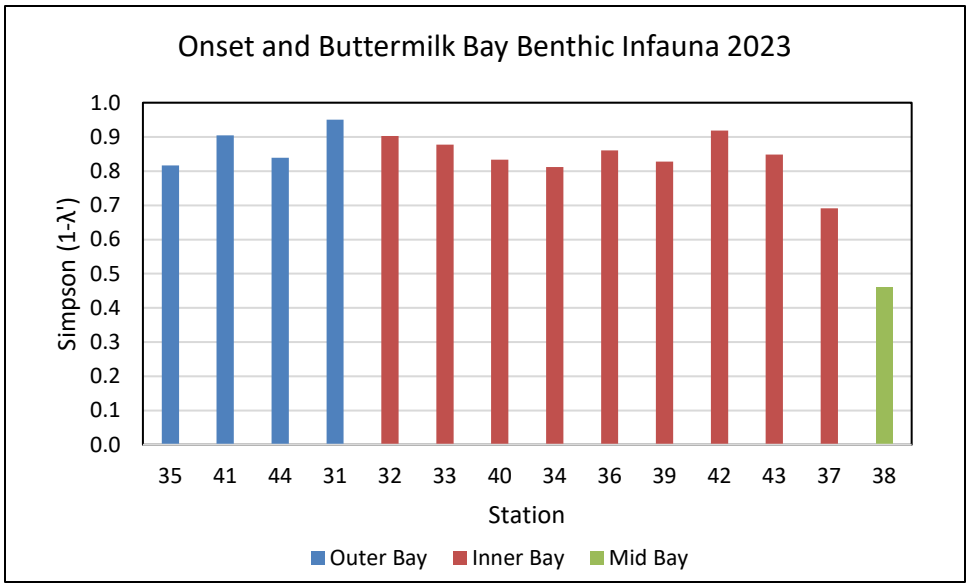


Figure 14. Simpson's diversity indices for the Onset and Buttermilk Bay System Benthos, 2023: Outer Bay (blue bars), Inner Bay (red bars), and Mid Bay (green bar).

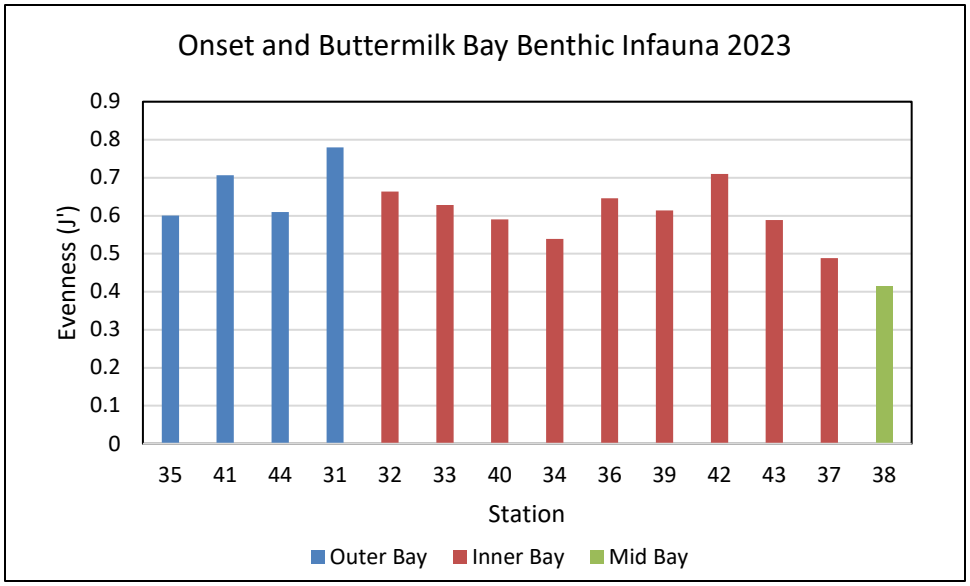


Figure 15. Pielou's evenness indices for the Onset and Buttermilk Bay System Benthos, 2023: Outer Bay (blue bars), Inner Bay (red bars), and Mid Bay (green bar).

3.4.5 Pollution-tolerant and Pollution-sensitive species

Macroinvertebrates are valuable indicators of pollution due to their relatively sedentary life history and predictable responses to contaminants and eutrophication pollution (Scott 1990, Pelletier et al. 2010). Pelletier et al. (2010) identified benthic invertebrates that could be used as indicator species to detect the presence (pollution-tolerant species) or absence (pollution sensitive species) of pollution for various habitats including polyhaline mud and polyhaline sand that are present in the Onset and Buttermilk Bay System. There were three pollution-sensitive indicator species identified: gastropod *Acteocina canaliculata* in the Inner Bay; polychaete *Alitta succinea* in the Inner Bay and Mid-Bay; and polychaete *Dipolydora socialis* in the Outer Bay and Inner Bay (Pelletier et al. 2010; Table 9). Although the presence of pollution-sensitive species is a sign of a healthy habitat, it should be noted that the abundance of these species was low, contributing less than 1% to 1% to the total per sample (Table 9).

Five pollution-tolerant species were identified: gastropod *Caecum pulchellum* in the Outer Bay, and four polychaetes, *C. capitata* complex in the Outer, Inner, and Mid Bay; *Dipolydora socialis* in the Outer and Inner Bay; *Marenzelleria* sp. in the Outer Bay; *Mediomastus ambiseta* at all stations in the Outer, Inner, and Mid Bay; and *Mediomastus californiensis* in the Outer and inner Bay (Table 9). Three of the species were recorded in relatively high percentages suggesting a stressed, low-quality habitat. *C. pulchellum* represented 10% of the taxa in Station MEP-SE-031; *C. capitata* complex contributed 11% at Station MEP-SE-042, 18% at Station MEP-SE-035, and 73% at Station MEP-SE-038; and *Mediomastus ambiseta* contributed 29% in Station MEP-SE-040 (Table 10).

Table 9. Pollution-sensitive and pollution-tolerant indicator species recorded in the Onset and Buttermilk Bay System, 2023.

Taxa	Taxonomic Group	Pollution sensitive/ tolerant	Habitat Type	Recorded in Station MEP-SE-	Subembayment in Onset and Buttermilk Bay System
Acteocina canaliculata	Gastropod	sensitive	polyhaline mud	32, 36, 37	Inner Bay
Alitta succinea	Polychaete	sensitive	polyhaline mud/sand	32, 34, 37, 38, 39	Inner and Mid Bay
Caecum pulchellum	Gastropod	tolerant	polyhaline sand	31	Outer Bay
Capitella capitata complex	Polychaete	tolerant	polyhaline mud/sand	31, 32, 33, 34, 35, 38, 39, 40,41, 42, 43, 44	Outer, Inner, and Mid Bay
Dipolydora socialis	Polychaete	sensitive	polyhaline mud/sand	31, 35, 40, 44	Outer and Inner Bay
Marenzelleria sp.	Polychaete	tolerant	polyhaline sand	41	Outer Bay
Mediomastus ambiseta	Polychaete	tolerant	polyhaline mud/sand	all stations	Outer, Inner, and Mid Bay
Mediomastus californiensis	Polychaete	tolerant	polyhaline mud/sand	31, 34, 41	Outer and Inner Bay

Table 10. Percentage of pollution-sensitive and pollution-tolerant species in the Onset and Buttermilk Bay benthic samples.

Pollution-sensitive species	Percent per Station (range)		
	Group 1	Group 2	Group 3
Acteocina canaliculata	NA	<1 to 1%	NA
Alitta succinea	NA	<1%	<1%
Dipolydora socialis	<1%	<1%	NA
Pollution-tolerant species			
Caecum pulchellum	10%	NA	NA
Capitella capitata complex	<1 to 18%	<1 to 11%	73%
Marenzelleria sp.	<1%	NA	NA
Mediomastus ambiseta	<1 to 2%	<1 to 29%	<1%
Mediomastus californiensis	<1%	4%	NA

Percent silt was superimposed on the MDS plot (Figure 16) to examine whether it was correlated with the relative benthic quality groupings indicated in the cluster and MDS plots. The correlation between the station location and percent silt was somewhat inconsistent. The percentage of silt in Group 1 stations tracked with the MDS plot with very low (1% at Station MEP-SE-031) to moderate (29% at Station MEP-SE-044). These four stations would be expected to have relatively low silt levels due to their location near the river mouth and corresponding greater tidal flushing compared to stations farther up the bays. The percent silt in Group 2 stations were highly variable ranging from low (5%) at Station MEP-SE-039 in Buttermilk Bay and MEP-SE-040 in upper Onset Bay to high (64%) at Station MEP-SE-032 in Broad Cove and (70%) at Station MEP-SE-043 in mid-Onset Bay. Station MEP-SE-032 is located high up in the System where tidal flushing is likely to be limited and MEP-SE-043 is located nearshore which may result in lower tidal flow and elevated sediment deposition. As mentioned above, the percent of silt in Group 3 Station MEP-SE-038 was high (67%) likely due to its location behind Onset Island, which may reduce the tidal flushing and increase sediment deposition.

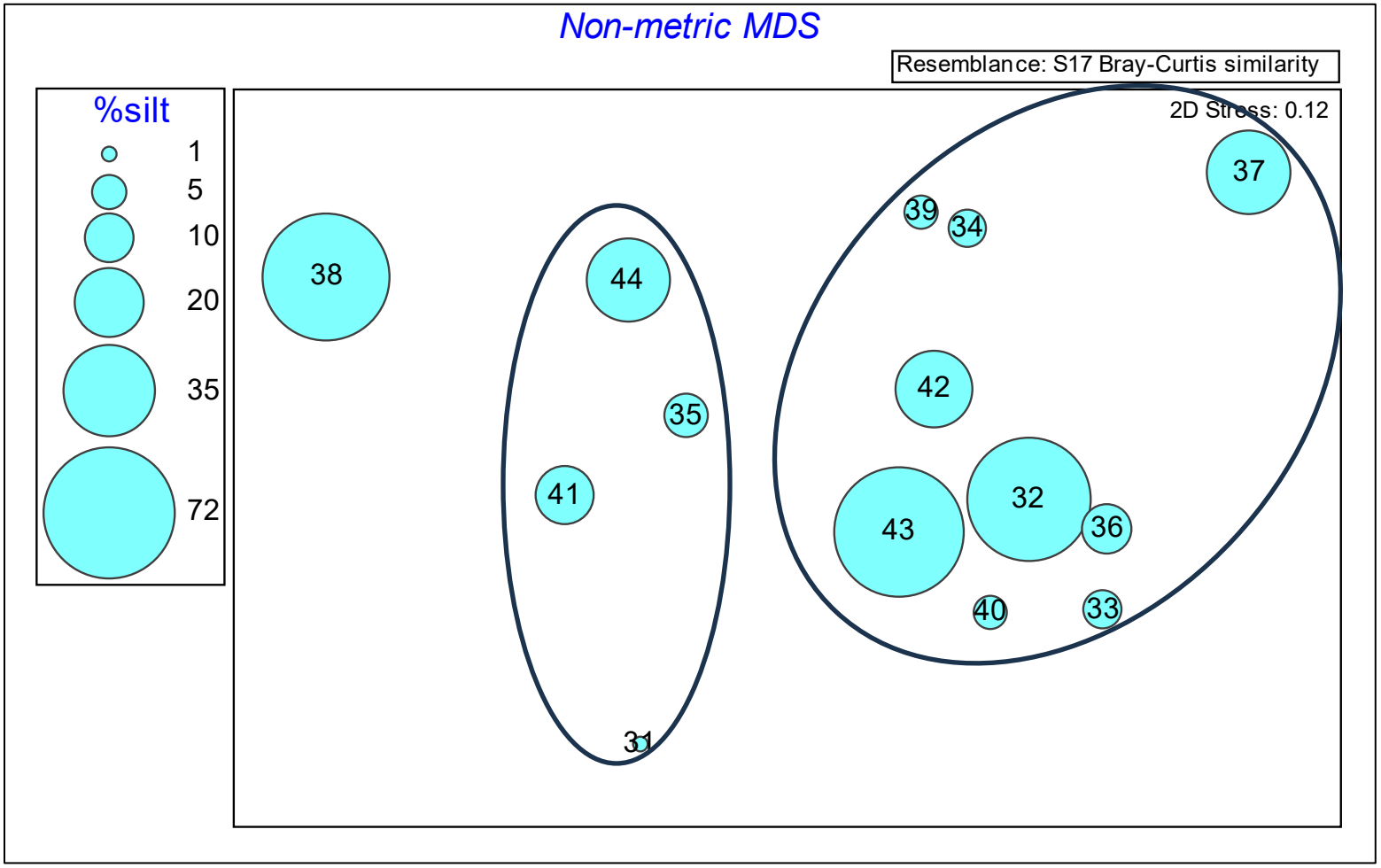


Figure 16. Percent fine sediments superimposed on the MDS ordination plot of the 2023 Onset and Buttermilk Bay infauna samples. Each point on the plot represents one of the 14 stations; similarity of species composition is indicated by proximity of points on the plot. Faunal assemblages (Groups 1 and 2) identified by cluster analysis are circled on the plot. The ordination and cluster analysis are both based on Bray-Curtis Similarity.

3.5 Average Taxonomic Distinctness (ATD)

Taxonomic distinctness is a biodiversity calculation used to indicate the relatedness of organisms based on Linnaean classification system. Average Taxonomic Distinctness (ATD) is a relatedness measure that can only be calculated from simple species lists (e.g. Phylum, Class, Order, Family, Genus, and Species) but also possesses a robustness to the varying number of species in the lists. More specifically, mean values are unchanged in different-sized sub-lists generated by random sampling from a larger list. This suggests that it is valid to compare Delta+ over historic time or biogeographic space scales, under conditions of variable sampling effort.

Taxonomic data for ATD analysis are required to be at the same classification level. In this data set several taxa were only identified to the genus level, therefore that was the lowest level analyzed. Subsequently, any taxa that were identified to the family, class, order, or phylum were not included in this analysis. Most of these upper-level taxa were represented by only a few specimens except Olicoghaeta, that were numerical dominants in several samples.

Average taxonomic distinctness (Delta+) for the Onset and Buttermilk Bay System benthos is represented in the funnel plot showing the 95% upper and lower limits of the expected range of diversity (Figure 17). Results indicate that while most samples are within the expected range, the following eight samples were below the expected range of biodiversity:

- Station MEP-SE-031 (replicate 3 presented in Figure 17 as 31-3),
- MEP-SE-032 (32-3),
- MEP-SE-033 (33-1),
- MEP-SE-033 (33-3),
- MEP-SE-035 (35-2),
- MEP-SE-040 (40-1),
- MEP-SE-040 (40-2), and
- MEP-SE-042 (42-3).

Data indicate that the relatively low diversity in these 8 samples are due to a combination of two factors: 1) Oligochaetes represented a relatively high percentage of the total abundance of 4 samples (11-42%), and the exclusion of oligochaetes negatively affected the abundance and corresponding diversity and/or numerical dominance of organisms from one or two phyla or orders. For example, four species of polychaetes contributed 71% to the total abundance in Sample MEP-SE-031-3. These ATD results appear to be slightly inconsistent with the other community parameters examined (i.e., Shannon- Weiner diversity and Pielou's evenness [Table 8]) for the above stations. However, since community parameters for each station were calculated using the mean of two samples, results by sample could be different. In addition, the removal of the upper-level taxa (i.e., those identified to family, class, order, or phylum) would also decrease the diversity in the ATD compared to the community parameters.

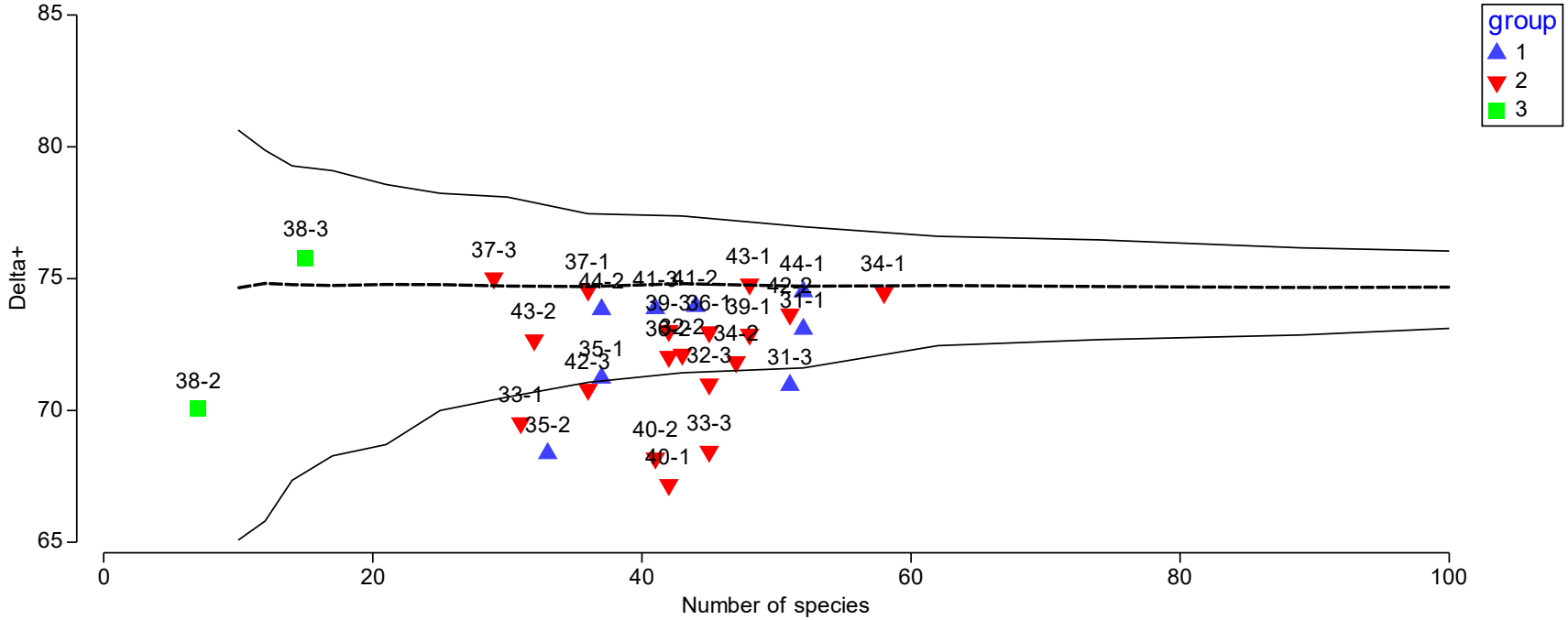


Figure 17. Onset and Buttermilk Bay Average Taxonomic Distinctness (Delta+) for all stations.

3.6 US M-AMBI

Overall, US M-AMBI results for the Onset and Buttermilk Bay System indicate that the benthic community is relatively healthy (Table 11, Figure 18). US M-AMBI scores in the Outer Bay (Group 1) ranged from Good (50% of the samples) to High (50% of the samples). Scores for both replicates at Station MEP-SE-035 nearshore on the western coast of Onset Island were classified as Good and both replicates at Station MEP-SE-031 were classified as High (Table 12). The scores for the other two stations in Group 1 (MEP-SE-041 and 044) differed among replicates and were classified as Good and High, respectively. In sample MEP-SE-041 -2 (replicate 2), the US M-AMBI score was at the high end of the Good range with a score of 0.76 (only 0.01 less than the High classification). Other parameters in Group 1 samples including percent silt and TOC were consistent with healthy benthic habitats (e.g., 1% to 29% silt and 0.3% - 0.9% TOC). These TOC levels also corresponded to Minimally Impaired benthic habitat based on Hyland et al. 2005 and Pelletier et al. 2011 studies (referred hereafter as the TOC indicator studies).

US M-AMBI results were similar in the Inner Bay (Group 2), ranging from Good (44% of the stations) to High (56% of the stations). For most stations both replicates were categorized the same. However, for two of the stations (MEP-SE-033 and 039), one replicate was categorized as Good and the other as High. In both cases, the Good scores were on the high end (0.70 and 0.73, respectively) of the range (0.53 – 0.77).

As described above, the percent silt within Group 2 was variable with moderate levels (5% - 29%) at all stations except Station MEP-SE-032 and 043 with high levels (64% and 70%, respectively). The US M-AMBI results for these two stations (both replicates for each station) were High/High and Good/Good, respectively. These results suggest that benthic habitats with high levels of silt are still capable of supporting relatively healthy benthic communities. TOC levels within Group 2 were generally low, ranging from 0.3% to 0.7% except Station MEP-SE-037 in Little Buttermilk Bay (3.7%). US M-AMBI results for both replicates of this sample (Good/Good) were inconsistent with the TOC indicator studies classification (Degraded).

Group 3 Station MEP-SE-038 replicate 2 and 3 in the mid-Onset Bay were classified as Bad (the lowest US M-AMBI category) and Poor (0.16 and 0.36, respectively). For context, a score >0.77 is classified as High habitat quality. For further clarification in this station, AMBI scores (5.7 [Heavily disturbed] and 4.6 [Moderately disturbed]; Table 12) were considered and were consistent with the US M-AMBI results. The high percentage of silt (67%) and pollution tolerant species (*C. capitata* complex, 73%) in addition to the strong sulfur odor noted by the field crew also supported these classifications. The only exception was the TOC, expected to be relatively high, was moderate (1.8%). Sampling indicated an impacted benthic community at station MEP-SE-038 and more sampling is needed to further elucidate the benthic health in this portion of Onset Bay (MA95-02).

Table 11. Summary of habitats in the Onset and Buttermilk Bay benthos, 2023.

Station	Group Name	Group Number	Sediment type from Survey Notes	% Silt	Salinity (ppt)	Salinity/Sediment category[1]	Depth (m)
35	Outer Bay	1	silty sand/sandy	8.0	29	polyhaline mud/sand	2.0
41			silty sand	14.2	30	polyhaline mud/sand	2.1
44			silty/sandy	29.0	30	polyhaline mud/sand	2.1
31			coarse sand	1.0	29-30	polyhaline sand	2.0
32	Inner Bay	2	fine, silty mud	63.7	29	polyhaline mud	1.5
33			sandy	6.2	28-29	polyhaline sand	0.9
40			sandy	4.7	29	polyhaline sand	2.0
36			fine, silty	10.3	25-28	polyhaline mud	1.0
43			fine and sandy	70.2	29-30	polyhaline mud/sand	3.2
42			silty sand	24.7	27	polyhaline mud/sand	0.5
34			silty sand	5.9	28	polyhaline mud/sand	0.5
39			silty sand	4.7	29	polyhaline mud/sand	0.5
37			soft mud	29.3	25-29	polyhaline mud	1.5
38	Middle Bay	3	silty, clumpy mud	67.5	28-30	polyhaline mud	3.0

1-Source: Pelletier et al. 2010.

Figure 18. Summary of US-M AMBI results for Onset and Buttermilk Bay benthos, 2023. Onset and Buttermilk Bay benthos, 2023. The circles at each station location represent qualitative AMBI scores Bad, Poor, Moderate, Good, and High for each station. Each circle represents the two replicate samples at each station, Replicate 1 on the left and Replicate 2 on the right half of each circle.

Table 12. US M-AMBI score and category for Onset and Buttermilk Bay benthic samples.

Sample	Group Name	Group Number	S	H'	% Oligochaetes	AMBI*	AMBI Category	US M-AMBI	US M-AMBI Category
MEP_SE_035_1	Outer Bay	1	39	2.49	29%	4.06	moderately disturbed	0.67	GOOD
MEP_SE_035_2			35	2.06	42%	4.55	moderately disturbed	0.56	GOOD
MEP_SE_041_2			47	2.75	26%	3.87	moderately disturbed	0.76	GOOD
MEP_SE_041_3			45	2.79	15%	2.68	slightly disturbed	0.84	HIGH
MEP_SE_044_1			58	2.51	27%	3.27	moderately disturbed	0.84	HIGH
MEP_SE_044_2			39	2.33	42%	3.91	moderately disturbed	0.66	GOOD
MEP_SE_031_1			56	3.16	5%	1.89	slightly disturbed	1.00	HIGH
MEP_SE_031_3			54	3.21	12%	2.00	slightly disturbed	1.00	HIGH
MEP_SE_032_2	Inner Bay	2	45	2.71	9%	3.38	moderately disturbed	0.78	HIGH
MEP_SE_032_3			48	2.62	11%	3.35	moderately disturbed	0.79	HIGH
MEP_SE_033_1			35	2.38	2%	3.17	slightly disturbed	0.70	GOOD
MEP_SE_033_3			47	2.51	1%	2.85	slightly disturbed	0.81	HIGH
MEP_SE_040_1			47	2.39	7%	3.36	moderately disturbed	0.76	GOOD
MEP_SE_040_2			43	2.27	7%	3.57	moderately disturbed	0.70	GOOD
MEP_SE_036_1			50	2.56	12%	2.61	slightly disturbed	0.85	HIGH
MEP_SE_036_2			45	2.58	8%	2.58	slightly disturbed	0.83	HIGH
MEP_SE_043_1			51	2.45	18%	4.10	moderately disturbed	0.74	GOOD
MEP_SE_043_2			35	2.19	15%	4.26	moderately disturbed	0.60	GOOD
MEP_SE_042_2			55	2.85	3%	2.98	slightly disturbed	0.89	HIGH
MEP_SE_042_3			41	2.81	10%	3.30	moderately disturbed	0.78	HIGH
MEP_SE_034_1			64	2.34	15%	2.01	slightly disturbed	0.95	HIGH
MEP_SE_034_2			55	2.30	22%	2.37	slightly disturbed	0.86	HIGH
MEP_SE_039_1			52	2.58	34%	3.33	moderately disturbed	0.81	HIGH
MEP_SE_039_3			45	2.36	42%	3.57	moderately disturbed	0.73	GOOD
MEP_SE_037_1			38	1.89	0.3%	2.29	slightly disturbed	0.72	GOOD
MEP_SE_037_3			31	1.64	1%	2.52	slightly disturbed	0.63	GOOD
MEP_SE_038_2	Mid Bay	3	8	0.69	4%	5.67	heavily disturbed	0.16	BAD
MEP_SE_038_3			17	1.35	1%	4.63	moderately disturbed	0.36	POOR

*AMBI = Calculated Biological Index (see methods section), S = number of taxa, N = number of individuals, H' = Shannon-Wiener diversity index

3.7 Current Factors Contributing to Habitat Health

Information on the Onset and Buttermilk Bay System benthic community health is relatively scarce. However, summaries of recent environmental data are provided in the MassDEP Embayment-specific Study Plan: Onset Bay and Buttermilk Bay System (MassDEP 2023d) and include the following:

- Final Integrated List of Waters for the Clean Water Act 2022 Reporting Cycle (MassDEP 2023),
- Shellfish classification areas: BB40- Onset Bay, MA; BB41- Sunset Cove (Onset), MA; BB42- East River System (Onset), MA; BB44- Buttermilk Bay (MDMF 2023),
- Buzzards Bay Coalition (BBC) Bay Health Index scores (BBC 2024),
- Upper Bay Regional Wastewater Feasibility Assessment Project (BBC 2022), and
- Stormwater and septic system remediation efforts for the reopening of shellfishing in Buttermilk Bay (BBNEP 2022), and
- B-120 Restoration Project shellfish out-planting and transfer (MDMF 2021)

The combination of these studies and this report indicate generally that the water quality and benthic habitat in Onset and Buttermilk Bay System are relatively healthy. However, the relative health status of the water quality and benthic habitat do not appear to track directly with the condition of eelgrass, which has been declining both locally and regionally since at least 1995 (MassMapper 2022). Eelgrass cover in the Onset and Buttermilk Bay System declined by 69% between 1995 and 2017 (MassDEP 2023c). Known primary causes of eelgrass loss to date include coastal development, declines in water quality, climate change, including rising water temperatures (Short and Wyllie-Echeverria 1996; Waycott et al. 2009; Wilson and Lotze 2019; Plaisted et al. 2022), and foraging green crabs (*Carcinus meanus*; Neckles 2015).

Nitrogen is one of the greatest threats to coastal water quality in the US (Costa 2013). Most of the nitrogen pollution that reaches Buzzards Bay comes from septic systems, wastewater treatment plants, and road runoff (Jakuba et al. 2023). The total nitrogen load in the Onset and Buttermilk Bay System is estimated to be approximately 41,998.6 kilogram per year (kg/yr), with about 21,736.3 kg/yr attributed to wastewater, 10,049.1 kg/yr to atmospheric deposition on the embayment, and 2,932.0 kg/yr to fertilizer. The total areal load of nitrogen in the Onset and Buttermilk Bay System is estimated to be 114.4 kilogram per hectare per year (kg/ha/yr; WHG 2021, MassDEP 2023c). Ninety-six percent of the Onset and Buttermilk Bays watershed (40,386 acres) is unsewered, relying on septic systems to treat wastewater (MassDEP 2023c).

Total nitrogen concentrations are relatively high in Buzzards Bay, including the Onset and Buttermilk Bay System. These concentrations range from 0.24 to 0.64 ppm (parts per million) in Onset Bay, 0.27 to 0.61 ppm in Buttermilk Bay, 0.30 to 1.11 ppm in Little Buttermilk Bay, and 0.35 to 0.62 ppm in Butler Cove over the past 30 years (BBC 2022). The BBC and Marine Biological Laboratory in Woods Hole have collected and analyzed nitrogen (organic and inorganic), dissolved oxygen, algal pigments, and water clarity data in several embayments in Buzzards Bay including the Onset and Buttermilk Bay System since 1992. The five parameters are summed into a single Bay Health Score ranging from 0 to 100 with a score of 0 indicating waters severely polluted with nitrogen and a score of 100 representing pristine waters. Scores from 0-35 are classed as Poor, 35-65 are Fair, and 65-100 are Good. While not used within the

regulatory framework for purposes of surface water quality standards attainment, Bay Health scores can provide context on system health.

Bay Health Scores for the Onset and Buttermilk Bay System range from Fair to Good. The most recent five-year average Bay Health Index scores in upper (Inner) Onset Bay (comparable to Stations MEP-SE-033, 032 and 040 from Group 2 in this report) ranged from 65-76, on the lower end of the Good range. These averages are calculated using data collected from 2018 to 2022. The Outer Onset Bay score (comparable to Group 1 Stations MEP-SE-035 and 041, Group 2 Station MEP-SE-042, and Group 3 Station MEP-SE-038) of 82 was ranked as Good, slightly lower than the prior five-year average (83; BBC 2024). The Bay Health Index score for Little Buttermilk Bay (comparable to Station MEP-SE-037 in Group 4) is lower than the upper estuary score, with a five-year average Index from 2018 through 2022 of 59 (Fair; a slight decline compared to the 2017 – 2021 average of 61). The Buttermilk Bay (comparable to Stations MEP-SE-034, 039, and 042 from Group 2) was categorized as Good with a five-year average score of 65, also a little lower compared to the prior five-year average of 69 (BBC 2024). Station MEP-SE-031 in Inner Cohasset Narrows was included in the BBC Buttermilk Bay Health Index Score.

Regardless of the relatively healthy benthic habitat in Onset and Buttermilk Bay, nitrogen is one of the greatest water quality concerns in this system and the region. In the 2000s, the Upper Bay Regional Wastewater Feasibility Assessment Project was formed with cooperation from several Buzzards Bay towns, including Bourne and Wareham. In addition to the studies and updates to the Wareham Water Pollution Control Facility (WPCF) reported in the Embayment-specific Study Plan (MassDEP 2023d), the town of Wareham and partnering Marine Biological Laboratory received several federal grants in October 2023 for over $315,000 to upgrade the experimental biofilters in use at the WPCF which have significantly improved effluent quality by lowering nitrogen levels in the discharge (CZM 2023). If successful, this new technology will allow an increased capacity at the WPCF as well as the expansion of sewering in the region, thus reducing overall nitrogen input by septic and wastewater sources. The Town of Bourne has also released a draft Comprehensive Wastewater Management Plan in May 2024, detailing the most recent nitrogen loading and reduction goals, as well as action steps to achieve those goals (Environmental Partners 2024).

Other recent actions have contributed to improvements in the water quality and benthic habitat in the Onset and Buttermilk Bay System. For example, quahog and oyster out-planting and reseeding efforts within the Buttermilk Bay System as part of the B-120 Shellfish Restoration Program from 2017-2020 were successful. Additional surveys were not needed and the study was concluded in 2021 (MDMF 2021). As further evidence of an improving Buttermilk Bay System, a shallow-water hard coral, Northern Star Coral (*Astrangia poculata*) was discovered in and area encompassing about 2.5 acres in Gibbs Narrows between Little Buttermilk Bay and Buttermilk Bay (CCT 2023). This coral is found from the Caribbean to as far north as Woods Hole, and on the west coast of Africa. It grows in clumps on hard surfaces, like rocks, piers, and oyster shells, but does not form reefs (MBL 2024). It is currently being studied by scientists to better understand how these corals can withstand a wide range of temperatures when most tropical corals are stressed by only a few degrees temperature change (MBL 2024).

Overall, despite relatively good benthic habitat health, there has been a decline in eelgrass over at least the past 30 years and efforts to restore eelgrass are needed.

4 Summary

The Onset and Buttermilk Bay System is a large, widespread embayment comprised of seven sub-embayments: Onset Bay, Shell Point Bay, Muddy Cove, Broad Cove, Cohasset Narrows, Buttermilk Bay, and Little Buttermilk Bay. Several biological and physical factors influence the benthic community assemblages and health status. Some factors include geographic location in the system and resulting restriction in tidal flushing, sediment deposition, and proximity to elevated nutrient concentrations. Overall, the US M-AMBI results indicated that benthic communities in the Onset and Buttermilk Bay System were healthy, regardless of whether the station was located in the Outer Bay (Group 1) or Inner Bay (Group 2). The exception was Station MEP-SE-038, approximately 250 m northeast of Onset Island in mid Onset Bay, which had a Bad/Poor benthic habitat score. All parameters measured and analyzed (except TOC, which was moderate) were consistent with the Bad/Poor US M-AMBI classification for this station. Additional benthic habitat sampling and sediment chemistry analysis may help identify the environmental stressors and its aerial extent in this location.

In addition to the US M-AMBI results, a summary of the health condition is provided in Table 13 based on the following seven factors: percent contribution from a single taxonomic group or species, present of pollution-sensitive or pollution-tolerant species, percent silt, percent TOC, AMBI classification, and US M-AMBI classification (Table 13). These results indicated that all station locations in the Onset and Buttermilk Bay System, except Station MEP-SE-038 in mid Onset Bay, had good to high quality benthic habitats independent of relative tidal flow.

Table 13. Summary of ecological data and benthic indices.

Group Number	Group Name	Maximum percent contribution from single taxonomic group or species (%)	Pollution Sensitive Species[2] (%)	Pollution Tolerant Species[2] (%)	Percent Silt (%) range	Percent TOC (%) range	TOC indicator[1]	AMBI (range)	US M-AMBI (range)
1	Outer Bay	25	<1	<1 - 18	1 - 29	0.3 - 0.9	Minimal impairment	1.9 - 4.6 Slightly - Moderately disturbed	0.56 - 1.00 Good - High
2	Inner Bay	15	<1 - 1	<1 - 29	5 - 70	0.3 - 3.7	Minimal impairment - Degraded	2.0 - 4.3 Slightly - Moderately disturbed	0.60 - 0.95 Good - High
3	Mid-Bay	73	<1	73	67	1.8	Moderate impairment	4.6 - 5.7 Moderately -Heavily disturbed	0.16 - 0.36 Poor - Bad

[1]Based on Hyland et al. 2005 and Pelletier et al. 2011.
[2]Percent of total abundance.

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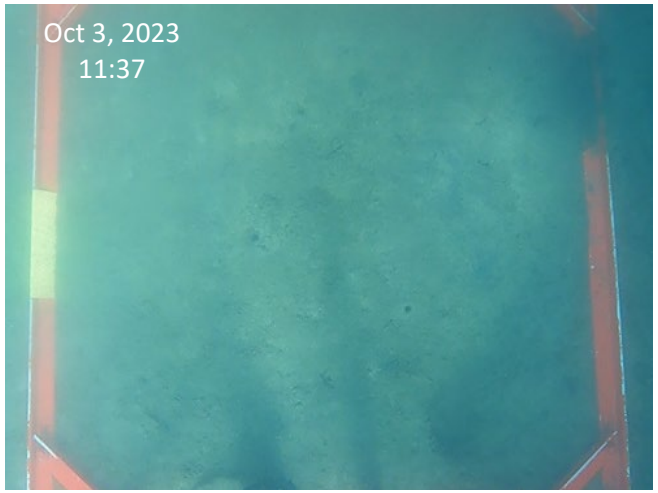
Appendix A. Water Quality Measurements in the Onset and Buttermilk Bay System, 2023

Station	AU-ID	Depth (m)	Temp (°C)	DO (mg/L)	pH	Salinity (ppt)
Onset Bay System						
MEP-SE-036	MA95-94	0.10	sample missing			
		0.50	17.91	7.38	7.61	25.26
		1.00	17.80	7.28	7.73	28.48
MEP-SE-033	MA95-108	0.10	sample missing			
		0.50	14.04	6.86	7.74	27.60
		0.90	14.38	7.03	7.86	28.86
MEP-SE-032	MA95-95	0.10	sample missing			
		0.50	18.26	7.35	7.94	28.59
		1.02	18.08	7.37	7.93	29.04
		1.55	17.76	7.64	7.98	29.26
MEP-SE-040	MA95-02	0.10	sample missing			
		0.50	17.77	7.14	7.90	29.27
		1.00	17.59	7.12	7.89	29.40
		2.02	17.34	7.06	7.90	29.56
MEP-SE-043	MA95-02	0.10	sample missing			
		0.51	17.76	7.23	7.93	28.94
		1.03	17.76	7.24	7.93	28.93
		2.00	17.43	7.26	7.95	29.69
		3.02	17.22	7.30	7.95	29.84
		3.20	17.18	7.33	7.95	29.87
MEP-SE-038	MA95-02	0.10	sample missing			
		0.51	18.15	7.36	7.99	27.65
		1.02	18.01	7.38	7.97	29.46
		2.01	17.94	7.41	7.97	29.47
		3.00	17.89	7.43	7.96	29.69
MEP-SE-035	MA95-02	0.10	sample missing			
		0.51	18.79	7.15	7.95	29.13
		1.01	18.17	7.18	7.95	29.28
		2.02	17.77	7.20	7.96	29.50
MEP-SE-041	MA95-02	0.10	sample missing			
		0.51	17.68	7.24	7.97	29.78
		1.02	17.64	7.27	7.97	29.89
		2.00	17.59	7.29	7.98	30.01
		2.14	17.58	7.33	7.98	30.02

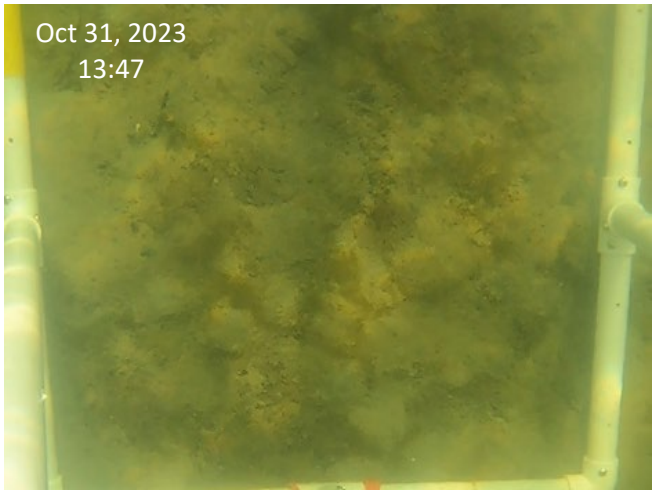
Station	AU-ID	Depth (m)	Temp (°C)	DO (mg/L)	pH	Salinity (ppt)
Buttermilk Bay System						
MEP-SE-037	MA 95-76	0.10	sample missing			
		0.51	14.58	8.11	7.75	25.02
		1.01	15.34	7.34	7.80	28.52
		1.49	15.41	7.40	7.91	28.68
MEP-SE-042	MA95-01	0.10	sample missing			
		0.50	15.09	8.08	7.96	27.39
MEP-SE-034	MA95-01	0.10	sample missing			
		0.51	15.98	8.32	8.08	28.07
MEP-SE-039	MA95-01	0.10	sample missing			
		0.50	15.13	7.97	8.02	29.00
MEP-SE-031	MA95-109	0.10	sample missing			
		0.50	17.78	6.87	7.88	29.44
		1.00	17.67	6.80	7.91	29.62
		2.01	17.66	6.80	7.91	29.62
MEP-SE-044	MA95-109	0.10	sample missing			
		0.50	17.54	7.27	7.97	29.77
		1.00	17.47	7.28	7.97	29.96
		2.06	17.45	7.30	7.98	29.96

Appendix B. Images of Soft Benthic Habitat from the Onset and Buttermilk Bay System 2023 Survey

a.



b.



c.

d.

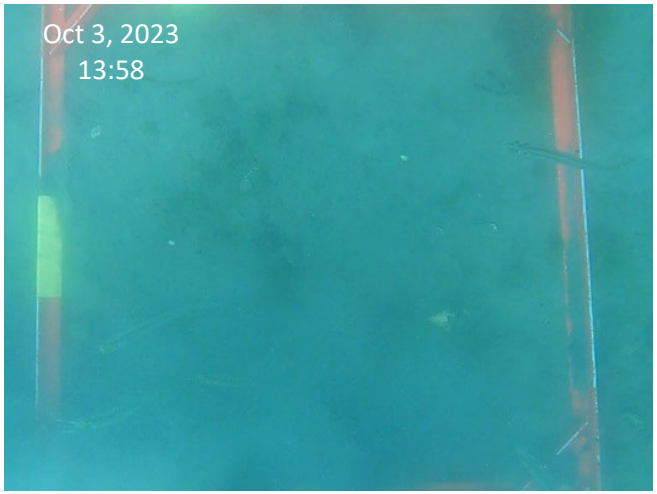


Figure B-1. Images of bottom habitat in the Onset inner Bay Stations: a) MEP-SE-036 in Shell Point Bay, b) MEP-SE-033 in Muddy Cove, and c & d) two images of MEP-SE-032 in East River/Broad Cove.

e.

f.

g.

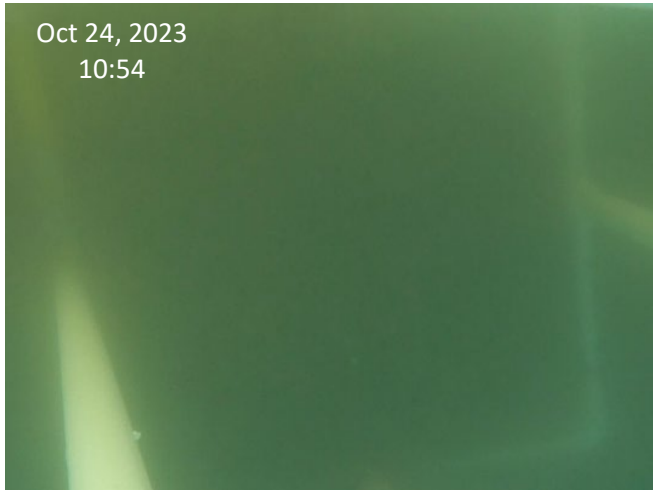
h.

Figure B-1. Continued. Images of bottom habitat in the mid- to outer-Onset Bay Stations: e) MEP-SE-040 f) MEP-SE-043, g) MEP-SE-038, and h) MEP-SE-035.

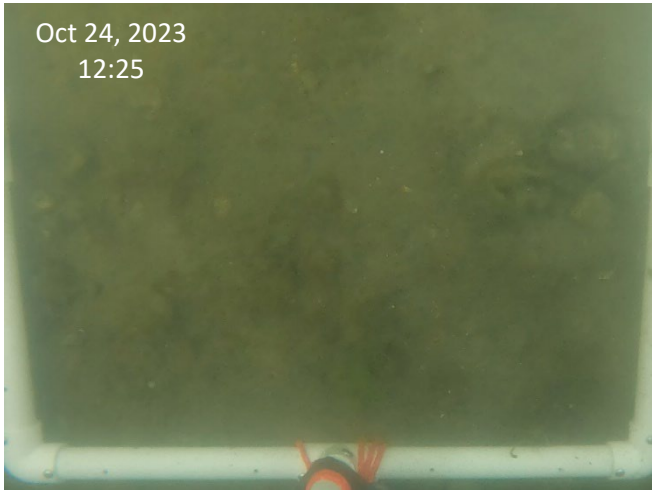
i. 

Figure B-1. Continued. i) Bottom habitat with sparse eel grass patches in the outer-Onset Bay Station MEP-SE-041.

a.



b.

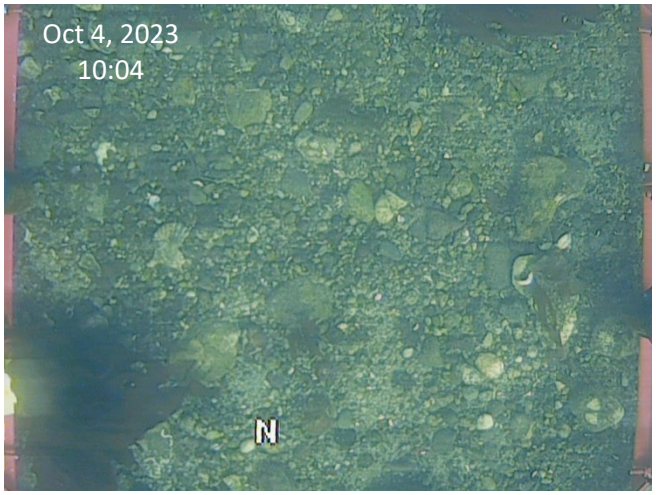


c.

d.

Figure B-2. Images of bottom habitat in the Buttermilk Bay Stations: a) MEP-SE-037 in Little Buttermilk Bay, b) MEP-SE-042 in inner Buttermilk Bay, c) MEP-SE-034 in Buttermilk Bay, and d) MEP-SE-039 in Buttermilk Bay.

e.



f.

Figure B-2. Continued. Bottom habitat in the outer-Buttermilk Bay System Stations: e) MEP-SE-031 and f) MEP-SE-044, both in Cohasset Narrows.

Appendix C. US M-AMBI Code Documentation

(continued next page)

MassDEP Massachusetts Estuaries Project (MEP) Benthic Monitoring Report

Onset and Buttermilk Bays 2023 Survey: M-AMBI Calculations

Normandeau Associates, Inc.

2024-10-09

Benthic Monitoring Report: Onset and Buttermilk Bays 2023 Survey

U.S. Multivariate AMBI (M-AMBI) Calculations

Prepared for: Massachusetts Department of Environmental Protection (MassDEP)

Prepared by: Normandeau Associates, Inc.

- *A. Villarreal 21 June 2024*
- *A. Villarreal 23 Aug 2024 - Revised Run (corrected BC and H1 input only)*
- *A. Villarreal 08 Oct 2024 - Revised Run (corrected BC; olig corrected to % instead of proportion, although not used for polyhaline)*

Resources:

- 'M-AMBI revisited: looking inside a widely-used benthic index' (Sigovini et al., 2013)
- 'Effect of ecological group classification schemes on performance of the AMBI benthic index in US coastal waters' (Gillett et al., 2015)
- 'Adaptation and application of multivariate AMBI (M-AMBI) in US coastal waters' (Pelletier et al., 2018)
- AZTI Marine Biotic Index species list
    - Updated June 2022
    - Downloaded May 2024
- New_mambi_script.R
    - Provided by Marguerite Pelletier on 17 May 2024

Comments:

- Original script for calculating M-AMB: mambisimpl.R
- The following code uses the updated M-AMBI script: New_mambi_script.R
    - Provided by Marguerite Pelletier on 17 May 2024
    - Does not depend on external packages to run M-AMBI

```
knitr::opts_chunk$set(echo = TRUE)
```

packages for markdown file creation

```
# packages for markdown file creation
library(knitr)
```

```
library(tinytex)
library(rmarkdown)
```

R version and session information

```
# session information
sessionInfo()

## R version 4.4.1 (2024-06-14 ucrt)
## Platform: x86_64-w64-mingw32/x64
## Running under: Windows 11 x64 (build 22631)
##
## Matrix products: default
##
##
## locale:
## [1] LC_COLLATE=English_United States.utf8
## [2] LC_CTYPE=English_United States.utf8
## [3] LC_MONETARY=English_United States.utf8
## [4] LC_NUMERIC=C
## [5] LC_TIME=English_United States.utf8
##
## time zone: America/New_York
## tzcode source: internal
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
## [1] rmarkdown_2.28 tinytex_0.53   knitr_1.48
##
## loaded via a namespace (and not attached):
##  [1] compiler_4.4.1    fastmap_1.2.0     cli_3.6.3         tools_4.4.1
##  [5] htmltools_0.5.8.1 rstudioapi_0.16.0 yaml_2.3.10       xfun_0.48
##  [9] digest_0.6.37     rlang_1.1.4       evaluate_1.0.0
```

setup and data import

```
# set working directory
setwd("I:\\MADEP\\Onset-Buttermilk Bay\\M_AMBI\\R")

# read in data
NCA_raw<-read.table("I:\\MADEP\\Onset-Buttermilk
Bay\\M_AMBI\\R\\OBB23_MAMBI_SAMPLE_IMPORT_REV2.csv", sep=",", header=TRUE)

# print input data
print.data.frame(NCA_raw)

##          Sample       BC  S        H1       olig Sbin_cd
## 1  MEP_SE_031_1 1.867159 56 3.1612303  5.2890529   P_RUS
## 2  MEP_SE_031_3 1.995261 54 3.2088926 11.8483412   P_RUS
## 3  MEP_SE_032_2 3.384512 45 2.7052918  9.1743119   P_RUS
## 4  MEP_SE_032_3 3.347166 48 2.6172408 10.9986505   P_RUS
## 5  MEP_SE_033_1 3.171021 35 2.3774990  1.6627078   P_RUS
## 6  MEP_SE_033_3 2.850490 47 2.5107317  1.2254902   P_RUS
## 7  MEP_SE_034_1 2.009664 64 2.3357995 14.9542218   P_RUS
## 8  MEP_SE_034_2 2.374659 55 2.2995200 21.7680896   P_RUS
## 9  MEP_SE_035_1 4.058036 39 2.4850288 28.7500000   P_RUS
## 10 MEP_SE_035_2 4.552174 35 2.0570233 41.7391304   P_RUS
## 11 MEP_SE_036_1 2.613881 50 2.5613510 11.7924528   P_RUS
## 12 MEP_SE_036_2 2.575121 45 2.5754209  7.5120606   P_RUS
```

```
## 13 MEP_SE_037_1 2.293017 38 1.8894336  0.3325021   P_RUS
## 14 MEP_SE_037_3 2.524845 31 1.6376143  1.1535049   P_RUS
## 15 MEP_SE_038_2 5.670732  8 0.6890438  3.6585366   P_RUS
## 16 MEP_SE_038_3 4.632353 17 1.3455314  0.4901961   P_RUS
## 17 MEP_SE_039_1 3.333776 52 2.5833274 34.3407506   P_RUS
## 18 MEP_SE_039_3 3.567278 45 2.3624719 42.2018349   P_RUS
## 19 MEP_SE_040_1 3.361463 47 2.3904225  6.8292683   P_RUS
## 20 MEP_SE_040_2 3.572120 43 2.2656872  6.5827686   P_RUS
## 21 MEP_SE_041_2 3.872979 47 2.7476646 25.6351039   P_RUS
## 22 MEP_SE_041_3 2.683377 45 2.7901568 14.5118734   P_RUS
## 23 MEP_SE_042_2 2.980921 55 2.8489029  3.0701754   P_RUS
## 24 MEP_SE_042_3 3.297669 41 2.8066175  9.9576271   P_RUS
## 25 MEP_SE_043_1 4.097859 51 2.4515341 17.9408767   P_RUS
## 26 MEP_SE_043_2 4.260000 35 2.1923676 15.3043478   P_RUS
## 27 MEP_SE_044_1 3.269001 58 2.5126825 27.4124680   P_RUS
## 28 MEP_SE_044_2 3.911765 39 2.3284752 42.2969188   P_RUS
```

salinity binning

```
#### salinity binning  ####

# TF_raw <- NCA_raw[which(NCA_raw$Sbin_cd=='TF'),]
# O_raw <- NCA_raw[which(NCA_raw$Sbin_cd=='O'),]
# M_raw <- NCA_raw[which(NCA_raw$Sbin_cd=='M'),]
P_RUS_raw <- NCA_raw[which(NCA_raw$Sbin_cd=='P_RUS'),]
# P_WEST_raw <- NCA_raw[which(NCA_raw$Sbin_cd=='P_WEST'),]
# E_RUS_raw <- NCA_raw[which(NCA_raw$Sbin_cd=='E_RUS'),]
# E_WEST_raw <- NCA_raw[which(NCA_raw$Sbin_cd=='E_WEST'),]
# Hyper_raw <- NCA_raw[which(NCA_raw$Sbin_cd=='Hyper'),]

# print binned data
# print(TF_raw)
# print(O_raw)
# print(M_raw)
print(P_RUS_raw)
```

```
##          Sample       BC  S        H1       olig Sbin_cd
## 1  MEP_SE_031_1 1.867159 56 3.1612303  5.2890529   P_RUS
## 2  MEP_SE_031_3 1.995261 54 3.2088926 11.8483412   P_RUS
## 3  MEP_SE_032_2 3.384512 45 2.7052918  9.1743119   P_RUS
## 4  MEP_SE_032_3 3.347166 48 2.6172408 10.9986505   P_RUS
## 5  MEP_SE_033_1 3.171021 35 2.3774990  1.6627078   P_RUS
## 6  MEP_SE_033_3 2.850490 47 2.5107317  1.2254902   P_RUS
## 7  MEP_SE_034_1 2.009664 64 2.3357995 14.9542218   P_RUS
## 8  MEP_SE_034_2 2.374659 55 2.2995200 21.7680896   P_RUS
## 9  MEP_SE_035_1 4.058036 39 2.4850288 28.7500000   P_RUS
## 10 MEP_SE_035_2 4.552174 35 2.0570233 41.7391304   P_RUS
## 11 MEP_SE_036_1 2.613881 50 2.5613510 11.7924528   P_RUS
## 12 MEP_SE_036_2 2.575121 45 2.5754209  7.5120606   P_RUS
## 13 MEP_SE_037_1 2.293017 38 1.8894336  0.3325021   P_RUS
## 14 MEP_SE_037_3 2.524845 31 1.6376143  1.1535049   P_RUS
## 15 MEP_SE_038_2 5.670732  8 0.6890438  3.6585366   P_RUS
## 16 MEP_SE_038_3 4.632353 17 1.3455314  0.4901961   P_RUS
## 17 MEP_SE_039_1 3.333776 52 2.5833274 34.3407506   P_RUS
## 18 MEP_SE_039_3 3.567278 45 2.3624719 42.2018349   P_RUS
## 19 MEP_SE_040_1 3.361463 47 2.3904225  6.8292683   P_RUS
## 20 MEP_SE_040_2 3.572120 43 2.2656872  6.5827686   P_RUS
## 21 MEP_SE_041_2 3.872979 47 2.7476646 25.6351039   P_RUS
## 22 MEP_SE_041_3 2.683377 45 2.7901568 14.5118734   P_RUS
## 23 MEP_SE_042_2 2.980921 55 2.8489029  3.0701754   P_RUS
## 24 MEP_SE_042_3 3.297669 41 2.8066175  9.9576271   P_RUS
```

```
## 25 MEP_SE_043_1 4.097859 51 2.4515341 17.9408767   P_RUS
## 26 MEP_SE_043_2 4.260000 35 2.1923676 15.3043478   P_RUS
## 27 MEP_SE_044_1 3.269001 58 2.5126825 27.4124680   P_RUS
## 28 MEP_SE_044_2 3.911765 39 2.3284752 42.2969188   P_RUS

# print(P_WEST_raw)
# print(E_RUS_raw)
# print(E_WEST_raw)
# print(Hyper_raw)
```

---

subset appropriate metrics and run M-AMBI

Tidal Freshwater

```
# ## Tidal Freshwater
# TF_model<-TF_raw[,-1]
# rownames(TF_model)<- TF_raw[,1]
# AMBI_var <- c("BC","olig","H1")
# metrics.ex <- TF_model[AMBI_var]
#
# good_TF<-c(0.15,0,1.93)
# bad_metric<-c(6,100,0)
#
# # Note: for the scores to be the right sign, and the eventual M-AMBI scores calculated
correctly, the bad metric needs to be above the good metric
# metrics.tot<-rbind(metrics.ex,bad_metric,good_TF)
# B_no<-nrow(metrics.tot)-1
# H_no<-nrow(metrics.tot)
# rownames(metrics.tot)[B_no]<-"B"
# rownames(metrics.tot)[H_no]<-"H"
#
# ## direct calculation, which produces the factor scores with the same signs of the scores
produced by the AZTI-Tecnalia AMBI software -from fun.mambisimple.R program but uses base
principle components analysis in R rather than psych
# options(warn = -1)
# METRICS.fa <- princomp(metrics.tot, cor = T, covmat = cov(metrics.tot))
# options(warn = 0)
# METRICS.fa.load <- loadings(METRICS.fa) %*% diag(METRICS.fa$sdev)
# METRICS.fa.load <- eigen(cor(metrics.tot))$vectors %*%
diag(sqrt(eigen(cor(metrics.tot))$values))
# METRICS.fa.load.varimax <- loadings(varimax(METRICS.fa.load))
# METRICS.scores <- scale(metrics.tot) %*% METRICS.fa.load.varimax
# colnames(METRICS.scores) <- c("x","y","z")
# METRICS.tr<-METRICS.scores*-1
#
# ## this code was pulled from the fun.mambisimple.R program. This projects the scores onto
the pollution vector
# EQR <- function(data) {
#   segm <- data[nrow(data),] - data[(nrow(data)-1),]
#   vett <- matrix(NA, nrow = nrow(data), ncol = ncol(data))
#   for (k in 1: ncol(data)) {vett[, k] <- data[(nrow(data)-1), k]}
#   vett <- data - vett
#   ris <- round((vett %*% segm / sqrt(sum(segm*segm))) / sqrt(sum(segm*segm)), 3)
#   return(ris)
# }
# eqr <- EQR(METRICS.tr)
# colnames(eqr)<- "M-AMBI"
#
# mambi_output <- cbind(metrics.tot,eqr,METRICS.tr)
```

```
# write.csv(mambi_output, "mambi_olig_TF.csv")
#
# # print m-ambi metrics
# print.data.frame(mambi_output)
```

Oligohaline

```
# ## Oligohaline
# O_model<-O_raw[,-1]
# rownames(O_model)<- O_raw[,1]
# AMBI_var <- c("BC","S","H1")
# metrics.ex <- O_model[AMBI_var]
#
# good_O<-c(0.53,16.0,2.12)
# bad_metric<-c(6,0,0)
#
# #Note: for the scores to be the right sign, and the eventual M-AMBI scores calculated
correctly, the bad metric needs to be above the good metric
# metrics.tot<-rbind(metrics.ex,bad_metric,good_O)
# B_no<-nrow(metrics.tot)-1
# H_no<-nrow(metrics.tot)
# rownames(metrics.tot)[B_no]<-"B"
# rownames(metrics.tot)[H_no]<-"H"
#
# ## direct calculation, which produces the factor scores with the same signs of the scores
produced by the AZTI-Tecnalia AMBI software
# options(warn = -1)
# METRICS.fa <- princomp(metrics.tot, cor = T, covmat = cov(metrics.tot))
# options(warn = 0)
# METRICS.fa.load <- loadings(METRICS.fa) %*% diag(METRICS.fa$sdev)
# METRICS.fa.load <- eigen(cor(metrics.tot))$vectors %*%
diag(sqrt(eigen(cor(metrics.tot))$values))
# METRICS.fa.load.varimax <- loadings(varimax(METRICS.fa.load))
# METRICS.scores <- scale(metrics.tot) %*% METRICS.fa.load.varimax
# colnames(METRICS.scores) <- c("x","y","z")
# METRICS.tr<-METRICS.scores*-1
#
# ## this code was pulled from the fun.mambisimple.R program
# EQR <- function(data) {
#   segm <- data[nrow(data),] - data[(nrow(data)-1),]
#   vett <- matrix(NA, nrow = nrow(data), ncol = ncol(data))
#   for (k in 1: ncol(data)) {vett[, k] <- data[(nrow(data)-1), k]}
#   vett <- data - vett
#   ris <- round((vett %*% segm / sqrt(sum(segm*segm))) / sqrt(sum(segm*segm)), 3)
#   return(ris)
# }
# eqr <- EQR(METRICS.tr)
# colnames(eqr)<- "M-AMBI"
#
# mambi_output <- cbind(metrics.tot,eqr,METRICS.tr)
# write.csv(mambi_output, "mambi_S_O.csv")
#
# # print m-ambi metrics
# print.data.frame(mambi_output)
```

Mesohaline

```
# ## Mesohaline
# M_model<-M_raw[,-1]
# rownames(M_model)<- M_raw[,1]
# AMBI_var <- c("BC","S","H1")
# metrics.ex <- M_model[AMBI_var]
```

```
#
# good_M<-c(0.85,26.0,2.48)
# bad_metric<-c(6,0,0)
#
# # Note: for the scores to be the right sign, and the eventual M-AMBI scores calculated
correctly, the bad metric needs to be above the good metric
# metrics.tot<-rbind(metrics.ex,bad_metric,good_M)
# B_no<-nrow(metrics.tot)-1
# H_no<-nrow(metrics.tot)
# rownames(metrics.tot)[B_no]<-"B"
# rownames(metrics.tot)[H_no]<-"H"
#
# ## direct calculation, which produces the factor scores with the same signs of the scores
produced by the AZTI-Tecnalia AMBI software
# options(warn = -1)
# METRICS.fa <- princomp(metrics.tot, cor = T, covmat = cov(metrics.tot))
# options(warn = 0)
# METRICS.fa.load <- loadings(METRICS.fa) %*% diag(METRICS.fa$sdev)
# METRICS.fa.load <- eigen(cor(metrics.tot))$vectors %*%
diag(sqrt(eigen(cor(metrics.tot))$values))
# METRICS.fa.load.varimax <- loadings(varimax(METRICS.fa.load))
# METRICS.scores <- scale(metrics.tot) %*% METRICS.fa.load.varimax
# colnames(METRICS.scores) <- c("x","y","z")
# METRICS.tr<-METRICS.scores*-1
#
# ## this code was pulled from the fun.mambisimple.R program
# EQR <- function(data) {
#   segm <- data[nrow(data),] - data[(nrow(data)-1),]
#   vett <- matrix(NA, nrow = nrow(data), ncol = ncol(data))
#   for (k in 1: ncol(data)) {vett[, k] <- data[(nrow(data)-1), k]}
#   vett <- data - vett
#   ris <- round((vett %*% segm / sqrt(sum(segm*segm))) / sqrt(sum(segm*segm)), 3)
#   return(ris)
# }
# eqr <- EQR(METRICS.tr)
# colnames(eqr)<- "M-AMBI"
#
# mambi_output <- cbind(metrics.tot,eqr,METRICS.tr)
# write.csv(mambi_output, "mambi_S_M.csv")
#
# # print m-ambi metrics
# print.data.frame(mambi_output)
```

Polyhaline-Rest of US

```
## Polyhaline-Rest of US
P_RUS_model<-P_RUS_raw[,-1]
rownames(P_RUS_model)<- P_RUS_raw[,1]
AMBI_var <- c("BC","S","H1")
metrics.ex <- P_RUS_model[AMBI_var]

good_P_RUS<-c(0.72,44.0,2.96)
bad_metric<-c(6,0,0)

# Note: for the scores to be the right sign, and the eventual M-AMBI scores calculated
correctly, the bad metric needs to be above the good metric
metrics.tot<-rbind(metrics.ex,bad_metric,good_P_RUS)
B_no<-nrow(metrics.tot)-1
H_no<-nrow(metrics.tot)
rownames(metrics.tot)[B_no]<-"B"
rownames(metrics.tot)[H_no]<-"H"
```

```r
## direct calculation, which produces the factor scores with the same signs of the scores
produced by the AZTI-Tecnalia AMBI software
options(warn = -1)
METRICS.fa <- princomp(metrics.tot, cor = T, covmat = cov(metrics.tot))
options(warn = 0)
METRICS.fa.load <- loadings(METRICS.fa) %*% diag(METRICS.fa$sdev)
METRICS.fa.load <- eigen(cor(metrics.tot))$vectors %*%
diag(sqrt(eigen(cor(metrics.tot))$values))
METRICS.fa.load.varimax <- loadings(varimax(METRICS.fa.load))
METRICS.scores <- scale(metrics.tot) %*% METRICS.fa.load.varimax
colnames(METRICS.scores) <- c("x","y","z")
METRICS.tr<-METRICS.scores*-1

## this code was pulled from the fun.mambisimple.R program
EQR <- function(data) {
  segm <- data[nrow(data),] - data[(nrow(data)-1),]
  vett <- matrix(NA, nrow = nrow(data), ncol = ncol(data))
  for (k in 1: ncol(data)) {vett[, k] <- data[(nrow(data)-1), k]}
  vett <- data - vett
  ris <- round((vett %*% segm / sqrt(sum(segm*segm))) / sqrt(sum(segm*segm)), 3)
  return(ris)
}
eqr <- EQR(METRICS.tr)
colnames(eqr)<- "M-AMBI"

mambi_output <- cbind(metrics.tot,eqr,METRICS.tr)
write.csv(mambi_output, "mambi_S_P_RUS_REV2.csv")

# print m-ambi metrics
print.data.frame(mambi_output)
```

```
##                     BC  S        H1 M-AMBI           x           y           z
## MEP_SE_031_1 1.867159 56 3.1612303  1.012  1.74709363  1.97533931 -1.85019703
## MEP_SE_031_3 1.995261 54 3.2088926  0.997  1.62726771  1.84418301 -1.80857846
## MEP_SE_032_2 3.384512 45 2.7052918  0.783  0.38769665  0.20287302 -0.52700906
## MEP_SE_032_3 3.347166 48 2.6172408  0.792  0.51416803  0.26414516 -0.52708620
## MEP_SE_033_1 3.171021 35 2.3774990  0.700 -0.35578969 -0.05966949  0.13599938
## MEP_SE_033_3 2.850490 47 2.5107317  0.809  0.52034482  0.58673773 -0.50086326
## MEP_SE_034_1 2.009664 64 2.3357995  0.947  1.62463395  1.62277213 -1.06667378
## MEP_SE_034_2 2.374659 55 2.2995200  0.864  0.97596466  1.07039005 -0.63237337
## MEP_SE_035_1 4.058036 39 2.4850288  0.672 -0.29279791 -0.62263002  0.11813835
## MEP_SE_035_2 4.552174 35 2.0570233  0.561 -0.94617514 -1.36126074  0.90219405
## MEP_SE_036_1 2.613881 50 2.5613510  0.850  0.79360351  0.88557173 -0.72380069
## MEP_SE_036_2 2.575121 45 2.5754209  0.825  0.52193474  0.79456479 -0.59000124
## MEP_SE_037_1 2.293017 38 1.8894336  0.720 -0.26661031  0.47337725  0.39265803
## MEP_SE_037_3 2.524845 31 1.6376143  0.632 -0.90596777 -0.03411208  0.98794979
## MEP_SE_038_2 5.670732  8 0.6890438  0.155 -3.73787339 -3.71574224  3.74153681
## MEP_SE_038_3 4.632353 17 1.3455314  0.363 -2.49211586 -2.27891764  2.36990882
## MEP_SE_039_1 3.333776 52 2.5833274  0.812  0.72851291  0.36104241 -0.61870593
## MEP_SE_039_3 3.567278 45 2.3624719  0.727  0.10933704 -0.13114149 -0.06058224
## MEP_SE_040_1 3.361463 47 2.3904225  0.757  0.30076150  0.10425913 -0.21527973
## MEP_SE_040_2 3.572120 43 2.2656872  0.704 -0.07314234 -0.23932671  0.12293189
## MEP_SE_041_2 3.872979 47 2.7476646  0.764  0.39933423 -0.12149448 -0.50983176
## MEP_SE_041_3 2.683377 45 2.7901568  0.844  0.63553108  0.82170983 -0.82139939
## MEP_SE_042_2 2.980921 55 2.8489029  0.888  1.17675904  0.87065796 -1.13455341
## MEP_SE_042_3 3.297669 41 2.8066175  0.778  0.24562755  0.22421197 -0.54464674
## MEP_SE_043_1 4.097859 51 2.4515341  0.735  0.37390268 -0.36057661 -0.21782564
## MEP_SE_043_2 4.260000 35 2.1923676  0.598 -0.77625872 -1.04954096  0.65802926
## MEP_SE_044_1 3.269001 58 2.5126825  0.843  1.04904127  0.53227118 -0.74429837
```

```
## MEP_SE_044_2 3.911765 39 2.3284752  0.664 -0.35724350 -0.58736857  0.26846758
## B            6.000000  0 0.0000000  0.000 -4.75356164 -4.56438936  4.92698577
## H            0.720000 44 2.9600000  1.000  1.22602128  2.49206375 -1.53109344
```

Polyhaline-West

```
# ## Polyhaline-West
# P_WEST_model<-P_WEST_raw[,-1]
# rownames(P_WEST_model)<- P_WEST_raw[,1]
# AMBI_var <- c("BC","S","H1")
# metrics.ex <- P_WEST_model[AMBI_var]
#
# good_P_WEST<-c(0.18,76.8,3.30)
# bad_metric<-c(6,0,0)
#
# # Note: for the scores to be the right sign, and the eventual M-AMBI scores calculated
correctly, the bad metric needs to be above the good metric
# metrics.tot<-rbind(metrics.ex,bad_metric,good_P_WEST)
# B_no<-nrow(metrics.tot)-1
# H_no<-nrow(metrics.tot)
# rownames(metrics.tot)[B_no]<-"B"
# rownames(metrics.tot)[H_no]<-"H"
#
# ## direct calculation, which produces the factor scores with the same signs of the scores
produced by the AZTI-Tecnalia AMBI software
# options(warn = -1)
# METRICS.fa <- princomp(metrics.tot, cor = T, covmat = cov(metrics.tot))
# options(warn = 0)
# METRICS.fa.load <- loadings(METRICS.fa) %*% diag(METRICS.fa$sdev)
# METRICS.fa.load <- eigen(cor(metrics.tot))$vectors %*%
diag(sqrt(eigen(cor(metrics.tot))$values))
# METRICS.fa.load.varimax <- loadings(varimax(METRICS.fa.load))
# METRICS.scores <- scale(metrics.tot) %*% METRICS.fa.load.varimax
# colnames(METRICS.scores) <- c("x","y","z")
# METRICS.tr<-METRICS.scores*-1
#
# ## this code was pulled from the fun.mambisimple.R program
# EQR <- function(data) {
#   segm <- data[nrow(data),] - data[(nrow(data)-1),]
#   vett <- matrix(NA, nrow = nrow(data), ncol = ncol(data))
#   for (k in 1: ncol(data)) {vett[, k] <- data[(nrow(data)-1), k]}
#   vett <- data - vett
#   ris <- round((vett %*% segm / sqrt(sum(segm*segm))) / sqrt(sum(segm*segm)), 3)
#   return(ris)
# }
# eqr <- EQR(METRICS.tr)
# colnames(eqr)<- "M-AMBI"
#
# mambi_output <- cbind(metrics.tot,eqr,METRICS.tr)
# write.csv(mambi_output, "mambi_S_P_WEST.csv")
#
# # print m-ambi metrics
# print.data.frame(mambi_output)
```

Euhaline-Rest of US

```
# ## Euhaline-Rest of US
# E_RUS_model<-E_RUS_raw[,-1]
# rownames(E_RUS_model)<- E_RUS_raw[,1]
# AMBI_var <- c("BC","S","H1")
# metrics.ex <- E_RUS_model[AMBI_var]
#
```

```
# good_E_RUS<-c(0.56,61.0,3.29)
# bad_metric<-c(6,0,0)
#
# # Note: for the scores to be the right sign, and the eventual M-AMBI scores  calculated
correctly, the bad metric needs to be above the good metric
# metrics.tot<-rbind(metrics.ex,bad_metric,good_E_RUS)
# B_no<-nrow(metrics.tot)-1
# H_no<-nrow(metrics.tot)
# rownames(metrics.tot)[B_no]<-"B"
# rownames(metrics.tot)[H_no]<-"H"
#
# ## direct calculation, which produces the factor scores with the same signs of the scores
produced by the AZTI-Tecnalia AMBI software
# options(warn = -1)
# METRICS.fa <- princomp(metrics.tot, cor = T, covmat = cov(metrics.tot))
# options(warn = 0)
# METRICS.fa.load <- loadings(METRICS.fa) %*% diag(METRICS.fa$sdev)
# METRICS.fa.load <- eigen(cor(metrics.tot))$vectors %*%
diag(sqrt(eigen(cor(metrics.tot))$values))
# METRICS.fa.load.varimax <- loadings(varimax(METRICS.fa.load))
# METRICS.scores <- scale(metrics.tot) %*% METRICS.fa.load.varimax
# colnames(METRICS.scores) <- c("x","y","z")
# METRICS.tr<-METRICS.scores*-1
#
# ## this code was pulled from the fun.mambisimple.R program
# EQR <- function(data) {
#   segm <- data[nrow(data),] - data[(nrow(data)-1),]
#   vett <- matrix(NA, nrow = nrow(data), ncol = ncol(data))
#   for (k in 1: ncol(data)) {vett[, k] <- data[(nrow(data)-1), k]}
#   vett <- data - vett
#   ris <- round((vett %*% segm / sqrt(sum(segm*segm))) / sqrt(sum(segm*segm)), 3)
#   return(ris)
# }
# eqr <- EQR(METRICS.tr)
# colnames(eqr)<- "M-AMBI"
#
# mambi_output <- cbind(metrics.tot,eqr,METRICS.tr)
# write.csv(mambi_output, "mambi_S_E_RUS.csv")
#
# # print m-ambi metrics
# print.data.frame(mambi_output)
```

Euhaline-West

```
# ## Euhaline-West
# E_WEST_model<-E_WEST_raw[,-1]
# rownames(E_WEST_model)<- E_WEST_raw[,1]
# AMBI_var <- c("BC","S","H1")
# metrics.ex <- E_WEST_model[AMBI_var]
#
# good_E_WEST<-c(0.66,92.0,3.62)
# bad_metric<-c(6,0,0)
#
# # Note: for the scores to be the right sign, and the eventual M-AMBI scorescalculated
correctly, the bad metric needs to be above the good metric
# metrics.tot<-rbind(metrics.ex,bad_metric,good_E_WEST)
# B_no<-nrow(metrics.tot)-1
# H_no<-nrow(metrics.tot)
# rownames(metrics.tot)[B_no]<-"B"
# rownames(metrics.tot)[H_no]<-"H"
#
```

```
# ## direct calculation, which produces the factor scores with the same signs of the scores
produced by the AZTI-Tecnalia AMBI software
# options(warn = -1)
# METRICS.fa <- princomp(metrics.tot, cor = T, covmat = cov(metrics.tot))
# options(warn = 0)
# METRICS.fa.load <- loadings(METRICS.fa) %*% diag(METRICS.fa$sdev)
# METRICS.fa.load <- eigen(cor(metrics.tot))$vectors %*%
diag(sqrt(eigen(cor(metrics.tot))$values))
# METRICS.fa.load.varimax <- loadings(varimax(METRICS.fa.load))
# METRICS.scores <- scale(metrics.tot) %*% METRICS.fa.load.varimax
# colnames(METRICS.scores) <- c("x","y","z")
# METRICS.tr<-METRICS.scores*-1
#
# ## this code was pulled from the fun.mambisimple.R program
# EQR <- function(data) {
#   segm <- data[nrow(data),] - data[(nrow(data)-1),]
#   vett <- matrix(NA, nrow = nrow(data), ncol = ncol(data))
#   for (k in 1: ncol(data)) {vett[, k] <- data[(nrow(data)-1), k]}
#   vett <- data - vett
#   ris <- round((vett %*% segm / sqrt(sum(segm*segm))) / sqrt(sum(segm*segm)), 3)
#   return(ris)
# }
# eqr <- EQR(METRICS.tr)
# colnames(eqr)<- "M-AMBI"
#
# mambi_output <- cbind(metrics.tot,eqr,METRICS.tr)
# write.csv(mambi_output, "mambi_S_E_WEST.csv")
#
# # print m-ambi metrics
# print.data.frame(mambi_output)
```

Hyperhaline

```
# ## Hyperhaline
# Hyper_model<-Hyper_raw[,-1]
# rownames(Hyper_model)<- Hyper_raw[,1]
# AMBI_var <- c("BC","S","H1")
# metrics.ex <- Hyper_model[AMBI_var]
#
# good_Hyper<-c(0.32,55.0,3.45)
# bad_metric<-c(6,0,0)
#
# # Note: for the scores to be the right sign, and the eventual M-AMBI scores calculated
correctly, the bad metric needs to be above the good metric
# metrics.tot<-rbind(metrics.ex,bad_metric,good_Hyper)
# B_no<-nrow(metrics.tot)-1
# H_no<-nrow(metrics.tot)
# rownames(metrics.tot)[B_no]<-"B"
# rownames(metrics.tot)[H_no]<-"H"
#
# ## direct calculation, which produces the factor scores with the same signs of the scores
produced by the AZTI-Tecnalia AMBI software
# options(warn = -1)
# METRICS.fa <- princomp(metrics.tot, cor = T, covmat = cov(metrics.tot))
# options(warn = 0)
# METRICS.fa.load <- loadings(METRICS.fa) %*% diag(METRICS.fa$sdev)
# METRICS.fa.load <- eigen(cor(metrics.tot))$vectors %*%
diag(sqrt(eigen(cor(metrics.tot))$values))
# METRICS.fa.load.varimax <- loadings(varimax(METRICS.fa.load))
# METRICS.scores <- scale(metrics.tot) %*% METRICS.fa.load.varimax
# colnames(METRICS.scores) <- c("x","y","z")
```

```
# METRICS.tr<-METRICS.scores*-1
#
# ## this code was pulled from the fun.mambisimple.R program
# EQR <- function(data) {
#   segm <- data[nrow(data),] - data[(nrow(data)-1),]
#   vett <- matrix(NA, nrow = nrow(data), ncol = ncol(data))
#   for (k in 1: ncol(data)) {vett[, k] <- data[(nrow(data)-1), k]}
#   vett <- data - vett
#   ris <- round((vett %*% segm / sqrt(sum(segm*segm))) / sqrt(sum(segm*segm)), 3)
#   return(ris)
# }
# eqr <- EQR(METRICS.tr)
# colnames(eqr)<- "M-AMBI"
#
# mambi_output <- cbind(metrics.tot,eqr,METRICS.tr)
# write.csv(mambi_output, "mambi_S_Hyper.csv")
#
# # print m-ambi metrics
# print.data.frame(mambi_output)
```