

# Summary of benthic conditions in the Three Bays estuary (Cape Cod, MA) as of 2019

Laura E Erban<sup>1</sup>, Donald J Cobb<sup>1</sup>, Charles S Strobel<sup>1</sup>, Casey K Tremper<sup>2</sup>, James D Hagy III<sup>1</sup>, Timothy R Gleason<sup>1</sup>

## Affiliations

<sup>1</sup> US EPA Office of Research and Development, Center for Environmental Modeling and Measurement, Atlantic Coastal Environmental Sciences Division, Narragansett RI

<sup>2</sup> ORAU, Oak Ridge TN

## Overview

Benthic (seafloor) habitat condition is an important indicator of the overall health of estuarine ecosystems. Estuaries are widely degraded on Cape Cod, in large part due to excess nutrients in wastewater, and many now have established Total Maximum Daily Loads (TMDL) for nitrogen. The Three Bays estuary (Barnstable, MA) has a nitrogen TMDL that calls for substantial load reductions to improve water quality and restore and maintain high quality benthic habitat (MassDEP, 2007). As part of TMDL development, a benthic survey was conducted in the early 2000s (Howes et al., 2006) under the Massachusetts Estuaries Project (MEP). This data summary documents a follow-up survey conducted in 2019 to update the condition assessment for Three Bays and establish a new baseline for evaluating the impact of planned watershed interventions, including sewerage, centralized wastewater treatment and alternative technologies, to reduce the load of nitrogen reaching the estuary.

The 2019 benthic survey of Three Bays included the prior stations as well as the TMDL sentinel station and additional randomized locations. These stations were visited in early September to make in-situ water quality measurements and collect sediment grabs. Grab samples were analyzed for grain size distribution, total organic carbon (TOC) content, and taxa-specific infaunal abundance. Infaunal data were assessed using the common ecological community metrics used in the prior survey (density, number of species, evenness and diversity). Overall benthic habitat condition was scored with the now widely used marine biotic index M-AMBI (Muxika et al., 2007; Sigovini et al., 2013) and associated condition classes for US coastal waters (Pelletier et al., 2018). Habitat condition was classified as ‘poor’ or worse at about 50% of stations (13 out of 25) surveyed in 2019. The worst conditions were found in enclosed upper areas of the estuary with better conditions in seaward subembayments.

These findings are consistent with the prior MEP survey but suggest that some additional degradation has occurred. The prior survey found moderate to significant impairment at 7 of the

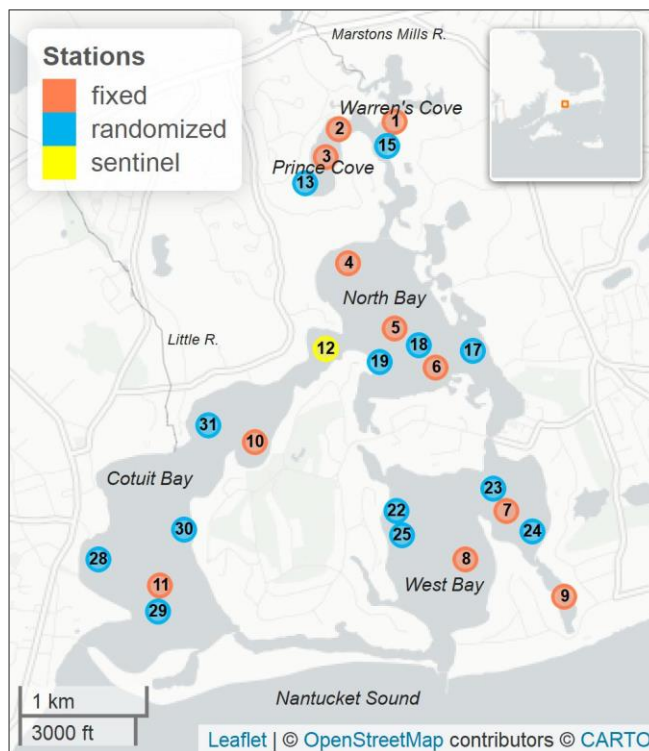
11 stations sampled. These remain impaired. Most (9) of the revisited stations show no discernable change in overall habitat condition while the remainder (2) are now assessed to be in worse condition. This is not unexpected, given that significant nutrient load reduction has yet to occur in the Three Bays watershed. In other local embayments, including West Falmouth Harbor, Pleasant Bay, and Wellfleet Harbor, similar surveys are being conducted under a pilot MEP monitoring study (see Sweeny and Rutecki, 2020). Results are being analyzed with comparable methods, including the quantitative summary of benthic condition provided by M-AMBI. This set of surveys will allow for assessment of changes in benthic habitat over time across Cape Cod estuaries subject to different baseline conditions and different management actions taken to address excess nutrients.

### Methods

The Three Bays estuary spans an area just over 5 km<sup>2</sup> on the south coast of Cape Cod, MA. Named for three interconnected major subembayments (North, West and Cotuit Bays), the northern end terminates in two smaller coves (Prince and Warren's). Flows from the Marstons Mills and Little Rivers contribute focused freshwater inputs (~20%, Howes et al., 2006), with the balance from direct precipitation and dispersed groundwater discharge. Tidal saltwater flow from Nantucket Sound (Atlantic Ocean) is exchanged through two southern inlets to Cotuit and West Bays. The average depth of the estuary as a whole is 2.3 m at mean tide level (computed from 1.9 m below NGVD 29 reported by Howes et al., 2006), including large areas that are more shallow and deeper navigational channels.

Benthic survey locations (Figure 1) included 11 fixed stations from the prior MEP survey, the TMDL sentinel station, and 13 new randomized sites. Randomized sites were selected using the Generalized Random Tessellation Stratified (GRTS) method, which generates spatially balanced, probabilistic survey designs for a given sample frame (Stevens and Olsen, 2004). Randomized sites, stratified by subembayment, were selected using the *spsurvey* package for R (Kincaid and Olsen, 2019). To develop a sample frame, or spatial representation of the target population to be sampled, data was acquired from NOAA's Electronic Navigational Charts

(<https://encdirect.noaa.gov/>, accessed 2019-04-18). GIS polygon layers were



**Figure 1.** The Three Bays estuary, subembayments, and locations of 2019 benthic survey stations.

downloaded for the 'Harbor' scale band for the extent of the estuary, which includes a depth contour for zero meters below Mean Lower Low Water (MLLW). The sample frame was limited to areas that are submerged (non-exposed) at MLLW.

Samples were collected from September 9-11, 2019 according to protocols previously developed for national coastal condition surveys (US EPA, 2015). At each station, in-situ water quality measurements (see Table 1) were made with a multi-parameter YSI EXO2 sonde at shallow and deep depths in the water column (or a single shallow depth, where the water column was <2m). Water depth recorded during sampling was used to calculate the mean tide level (MTL) depth by referencing levels recorded by the NOAA tide gauge at Nantucket adjusted by tidal phase lags from Howes et al. (2006) and local tidal datums obtained from the NOAA VDATUM tool (<http://vdatum.noaa.gov>). Secchi depth was recorded and used to estimate the fraction of incident light reaching sediments at MTL. Sediment grabs were recovered with a 0.04 m<sup>2</sup> Young modified Van Veen grab sampler. Acceptable grabs were at least 7 cm deep with a level surface. The first sediment grab, collected for infauna, was sieved on board using ambient seawater and a 0.5 mm screen. All material retained was transferred to a 1 L Nalgene bottle and fixed in 10% buffered formalin with Rose Bengal stain. The second grab, collected for sediment properties, was subsampled (~100 cm<sup>3</sup>) and stored at 4°C prior to analysis.

Infaunal community composition and sediment properties were analyzed by Normandeau Associates, Inc. and Alpha Analytical, respectively, following established procedures (US EPA, 1995). Briefly, the sieved infaunal sample was sorted under a dissecting microscope and macroinvertebrates were enumerated and identified by taxonomic group, to the species level when possible. Sediments were sorted to determine grain size distribution with a set of standard sieves, and hydrometer for fine fractions, according to ASTM D6913/D7928, and classified as per the Unified Soil Classification System (USCS). Acidified sediment samples were analyzed for total organic carbon (TOC) content as in US EPA 9060A, using a Perkin Elmer 2400 Series II CHNS/O Analyzer with a thermal conductivity detector. Additional details of the analyses performed by both contractors and their results are provided in the supplemental materials, available at [doi:10.23719/1520968](https://doi.org/10.23719/1520968). Field and laboratory data will also be made available in the [Water Quality Portal](#) to support their broader discoverability.

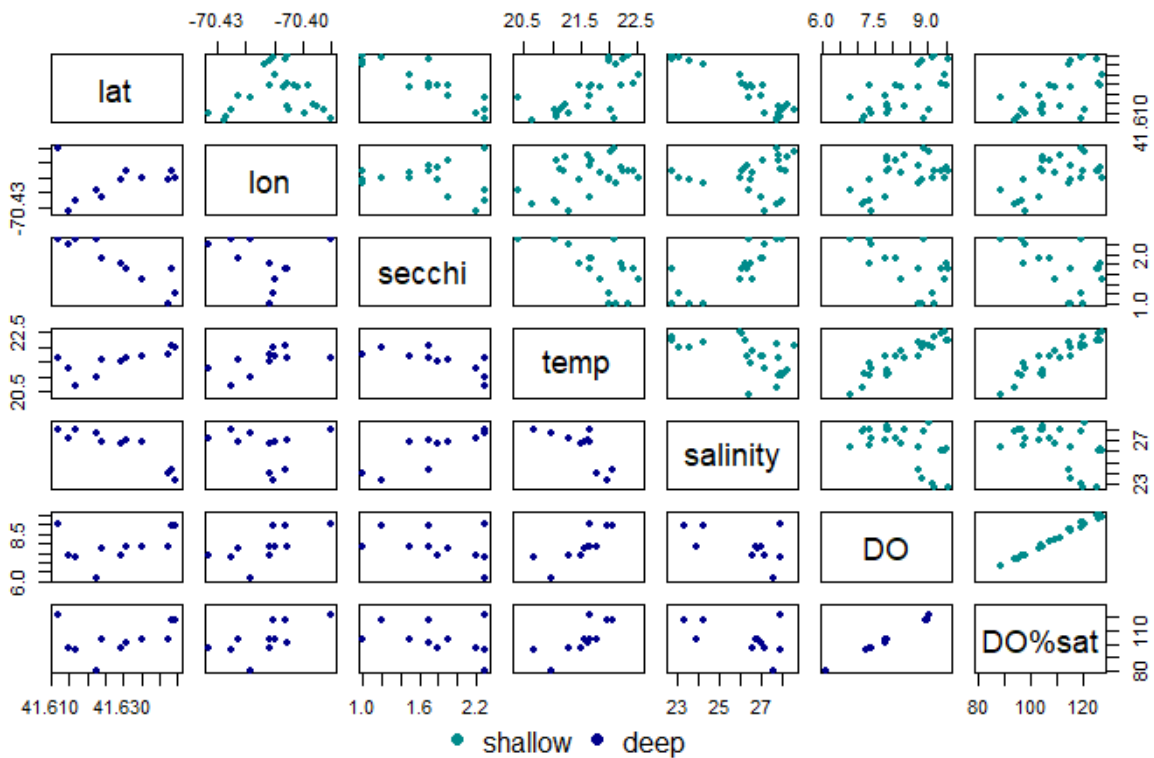
Computational analysis is coded in the supplemental html notebook, which includes interactive versions of the maps shown here and R packages used. Specialized packages *oce* (Kelley and Richards, 2020) and *vegan* (Oksanen et al., 2019) were used to calculate relative seawater densities and infaunal community metrics. Infaunal taxa were assigned to ecological groups for US coastal waters (hybrid values from Gillett et al., 2015). The AZTI Marine Biotic Index (AMBI; Borja et al., 2000) was calculated according to the representation of individuals in each group. Multivariate AMBI scores (M-AMBI; Muxika, et al., 2007) for the salinity bin (polyhaline) for these samples were calculated following Sigovini et al., (2013). M-AMBI was not calculated for any station where more than 50% of the sample abundance could not be assigned to an ecological group (these were typically oligochaetes, or worms). Established values (see Pelletier et al., 2018) were used for the reference anchor points for the Northeast USA and cut-points for condition classes (Bad, Poor, Moderate, Good, High).

**Table 1.** Water quality observations at surveyed stations by type and subembayment. Depths are for the measured water column at the time of sampling. A Secchi depth measurement of “NA” indicates that the disk could be seen on the bottom.

Subembayment	Station	Type	Date	Time	Latitude	Longitude	Depth (m)	Secchi depth (m)	Obs. depth (m)	Temp. (°C)	Salinity (ppt)	DO (mg/L)	DO (% <i>sat</i> )
Warren's Cove	1	<i>fixed</i>	9/11/2019	12:27	41.644967	-70.40557	1.0	1	0.5	22.3	22.8	9.2	120.3
Prince Cove	2		9/11/2019	11:29	41.644417	-70.41062	2.4	1.2	1.0	22.0	23.1	9.1	119.6
									2.0	22.0	23.4	9.0	117.1
	3		9/11/2019	11:08	41.642533	-70.41185	2.2	1	1.0	22.0	23.6	8.8	115.7
									2.0	21.8	24.0	7.8	103.5
North Bay	4		9/10/2019	13:48	41.635167	-70.4099	4.2	1.5	1.0	22.5	26.0	9.5	127.4
									3.0	21.7	26.8	7.8	103.9
	5		9/10/2019	14:13	41.630767	-70.40547	3.6	1.7	1.0	22.4	26.1	9.4	126.2
									3.0	21.6	27.1	7.8	100.5
	6		9/11/2019	9:39	41.62805	-70.40173	2.0	1.7	1.0	21.7	26.3	8.7	115.4
West Bay	7		9/10/2019	11:11	41.618117	-70.39522	1.4	NA	1.0	21.6	27.9	8.3	111.4
	8		9/10/2019	10:09	41.614833	-70.3991	1.4	NA	1.0	21.1	27.9	7.9	104.4
Eel River	9		9/10/2019	12:25	41.612217	-70.39	2.4	2.3	1.0	22.1	27.8	8.9	119.4
									2.0	21.7	28.0	9.0	120.7
Cotuit Bay	10		9/9/2019	9:20	41.622867	-70.41848	2.5	2.3	0.5	20.4	26.4	6.8	88.2
									2.0	21.0	27.6	6.1	80.3
	11		9/9/2019	11:17	41.612967	-70.42712	1.7	NA	1.0	21.1	27.9	7.2	95
	12	<i>sentinel</i>	9/11/2019	8:25	41.62925	-70.41192	2.8	1.8	1.0	21.5	26.5	7.4	97.4
									2.5	21.5	26.6	7.4	97.3
Prince Cove	13	<i>randomized</i>	9/11/2019	10:46	41.640767	-70.41375	1.7	1	1.0	22.1	24.2	8.8	115.1
Warren's Cove	15		9/11/2019	12:54	41.6433	-70.40618	2.4	1.7	1.0	22.2	22.7	9.6	125.3
									2.0	22.1	24.3	8.9	117.8
North Bay	17		9/11/2019	10:10	41.629233	-70.39833	1.9	1.9	1.0	21.7	27.1	8.1	107.6
	18		9/10/2019	14:35	41.629617	-70.40332	1.9	1.8	1.0	22.2	26.3	9.5	127.2
	19		9/11/2019	9:05	41.6284	-70.4069	1.6	1.5	1.0	21.8	26.6	8.2	109.7
West Bay	22		9/10/2019	9:07	41.618183	-70.40535	1.4	NA	1.0	21.2	28.2	7.9	104.4
	23		9/10/2019	10:43	41.619733	-70.39637	1.4	NA	1.0	21.2	28.3	7.9	104.6
	24		9/10/2019	11:41	41.61675	-70.39278	1.3	NA	1.0	22.0	28.6	9.0	121.2
	25		9/10/2019	9:38	41.616417	-70.40482	1.7	NA	1.0	21.1	27.9	7.9	104.1
Cotuit Bay	28		9/9/2019	12:38	41.6148	-70.43288	2.6	2.2	0.5	21.3	27.2	7.4	97.9
									2.0	21.3	27.2	7.4	97.4
	29		9/9/2019	10:25	41.611183	-70.42737	1.8	NA	1.0	20.6	27.8	7.2	94
	30		9/9/2019	13:18	41.616833	-70.42497	3.1	2.3	1.0	21.0	28.0	7.3	96.7
									2.6	20.7	28.0	7.3	95.6
	31		9/9/2019	14:04	41.624	-70.42278	2.6	1.9	0.5	21.7	27.0	7.8	103.4
									2.0	21.6	26.9	7.8	103.2

## Results and Discussion

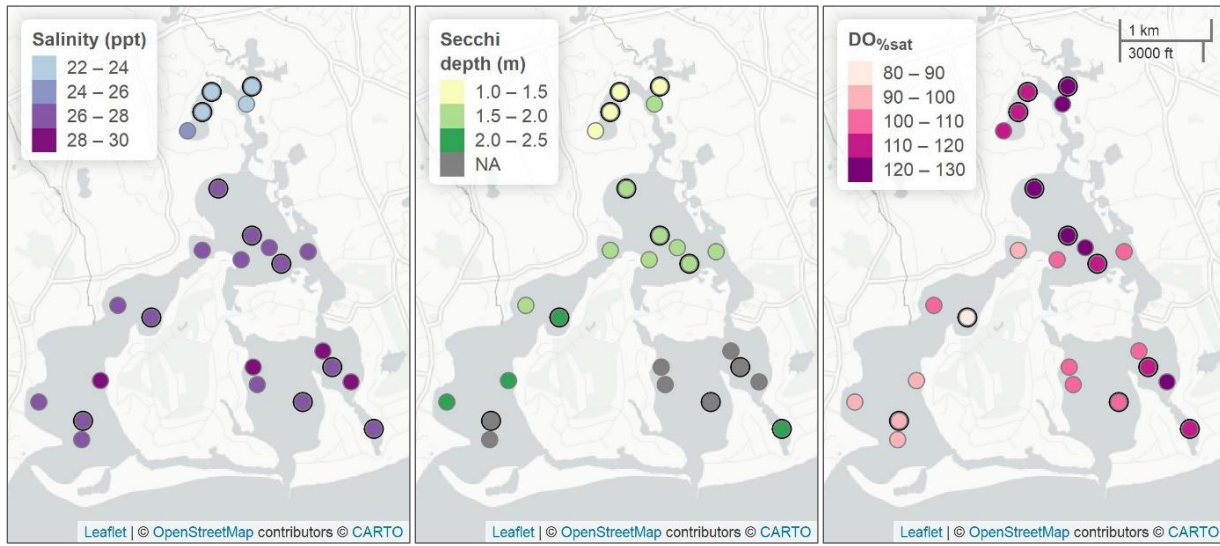
Water quality data from surveyed stations (Table 1) indicate generally well-mixed, euphotic conditions. Surface salinities of 22.7-28.6 ppt reflect significant tidal flushing relative to freshwater input. The freshwater fraction in the estuary was limited to between 5 and 25%, given that offshore salinity measured at the Waquoit Bay National Estuarine Research Reserve was 30.2 ppt during the survey. Salinity in the Three Bays increased linearly from north to south as did water clarity (Figures 2-3). Secchi depth (s.d.) measurements ranged from 1 to 2.3m where they could be made; the disk was visible on the bottom at 8 of 25 sites despite the water level being above mean tide level (MTL) when most stations were sampled. Assuming the measured Secchi depth was a lower limit when the disk was visible on the bottom, the minimum light fraction (assuming  $K_d=1.45/s.d.$ , Batiuk et al., 1992) reaching sediments at those sites at MTL was 31 to 37%. At sites where the Secchi disk was not visible on the bottom, the light fraction reaching sediments at MTL was 2-40% (mean=18%). Across all stations the median light fraction reaching sediments at MTL was 24%.



**Figure 2.** Scatterplots of water quality parameters at sampled stations. Shallow measurements (0.5-1m) are plotted above the diagonal, deep measurements (2-3m) are plotted below it. Units for each parameter correspond to those given in Table 1.

Vertical differences in salinity, temperature, and density were minimal; maximum absolute values were S: 1.6 ppt, T: 0.9°C,  $\Delta\sigma_t$ : 1.2. Vertical differences (shallow-deep) in dissolved

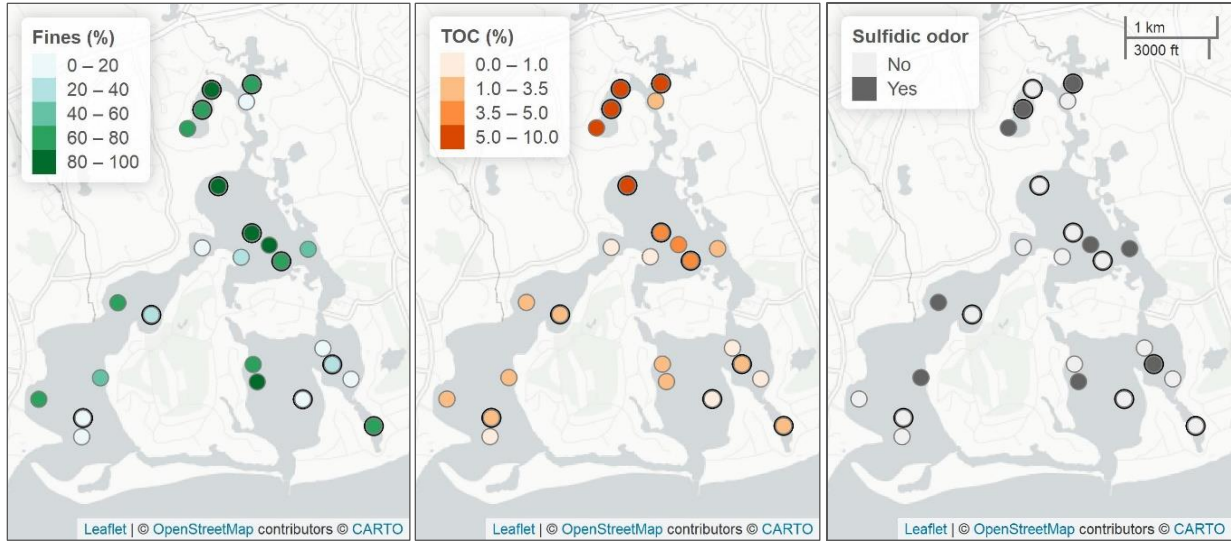
oxygen (DO) varied from -0.2 to 1.6 mg/L (-1.3 to 26%) and were largest at the two deepest sites, both in North Bay. The minimum observed DO was 6.1 mg/L; actual minimum values likely occurred overnight, however, not during the day when these measurements were made. DO saturation ranged from 80-127% (median: 105%). Supersaturation of oxygen at shallow water sites (0.5-1m), likely due to in large part to benthic primary production, was observed throughout the northern subembayments and West Bay.



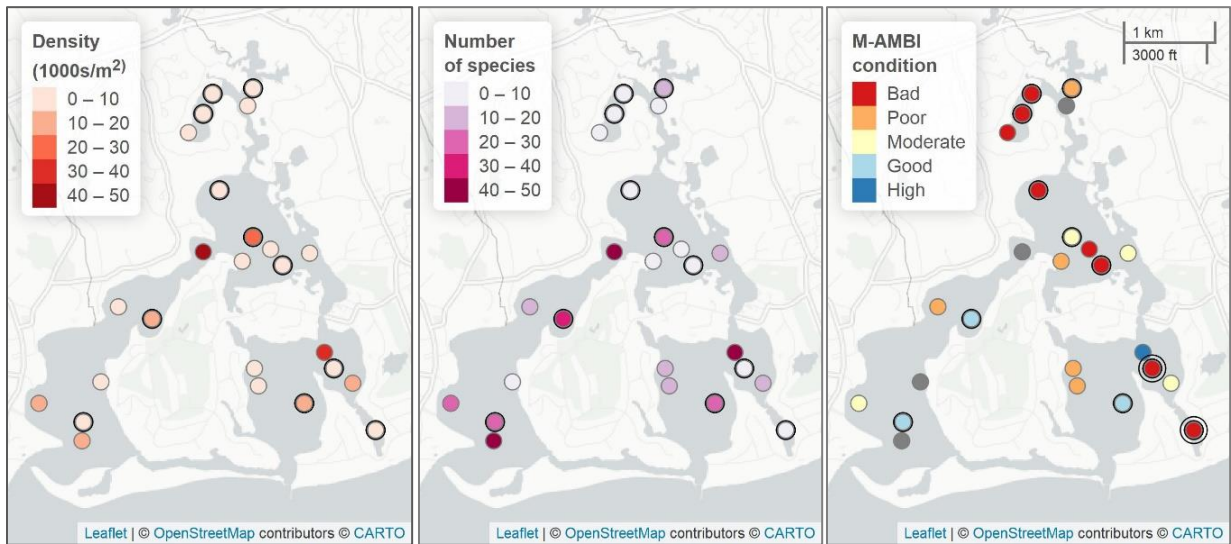
**Figure 3.** Maps of selected water quality measures. The salinity and dissolved oxygen saturation ( $DO_{\%sat}$ ) maps were made with the shallow (0.5-1m) observations, which were made at all stations. Secchi depths shown in gray indicate that the disk could be seen on the bottom. Fixed stations are outlined in black.

Grabbed sediments were typically black or brown with significant fines. More than half of the samples had a combined silt and clay (i.e., fines) content greater than 60% (see Table 2). The balance was largely sand, with minimal gravels (max: 5.2% of sample). The sediment had a sulfidic odor at 9 out of 25 sites with no clear spatial pattern (Figure 4). The total organic carbon (TOC) content in sediments, which varied inversely with salinity, ranged from <1% to 9.5% (median: 2.8%). These levels are considered intermediate (>1% TOC) and high (>3.5% TOC) at 76% and 32% of stations, respectively, compared with sediments globally (Hyland et al., 2005). TOC enrichment can also be assessed using a region-specific relationship with grain size (see Pelletier et al., 2011, Virginian province). Measured TOC was higher than predicted by this relationship at most stations (24 of 25), by a factor of 2.6, on average.





**Figure 4.** Maps of selected sediment properties: percent fines (silt and clay), total organic carbon (TOC) content, and field-noted sulfidic odor. Fixed stations are outlined in black.



**Figure 5.** Maps of benthic infaunal density, number of species, and M-AMBI condition. Fixed stations are outlined in black. M-AMBI was not calculated at the stations shown in gray due to overrepresentation of individuals that could not be assigned to an ecological group. M-AMBI scores were assigned to condition classes for US coastal waters as follows (from Pelletier et al., 2018): Bad (<0.2), Poor (0.2-0.39), Moderate (0.39-0.53), Good (0.53-0.77), High (>0.77). Locations where habitat condition has worsened since the prior survey are indicated with a double outline.

**Table 2.** Sediment properties at surveyed stations. ND = not detected.

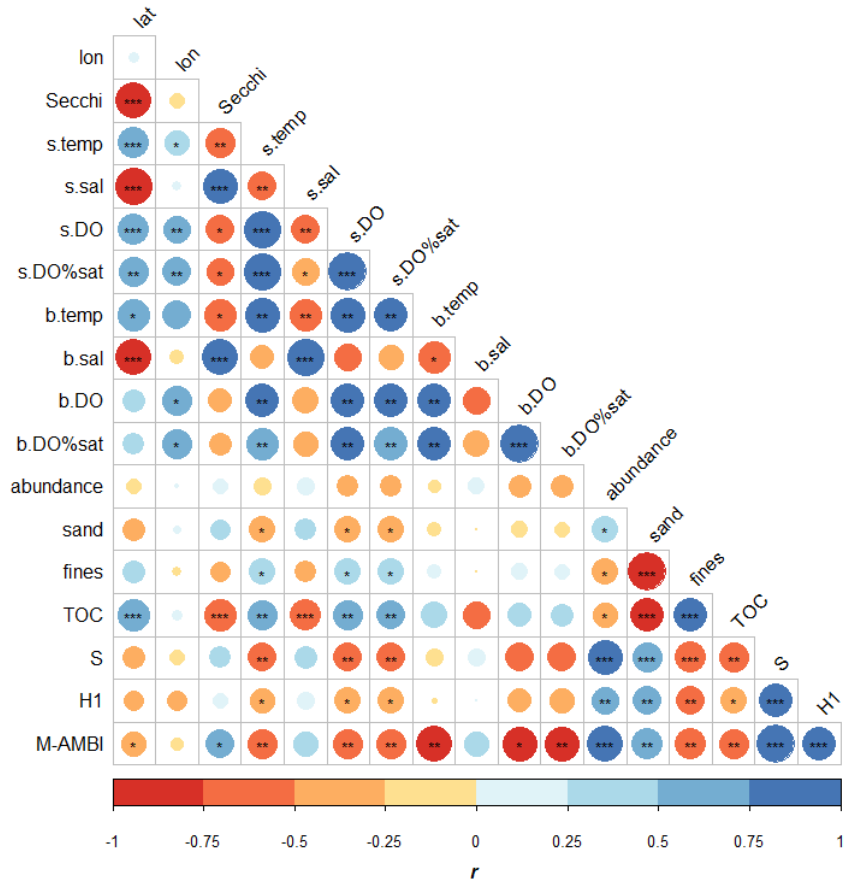
<b>Subembayment</b>	<b>Station</b>	<b>Gravel (%)</b>	<b>Sand (%)</b>	<b>Fines (%)</b>	<b>TOC (%)</b>	<b>Sulfidic odor</b>
Warren's Cove	1	ND	25.6	74.4	9.5	Yes
Prince Cove	2	ND	12.5	87.5	6.4	No
	3	ND	25.1	74.9	6.7	Yes
North Bay	4	ND	5.3	94.7	5.3	No
	5	ND	7.2	92.8	4.9	No
	6	ND	24.2	75.8	3.8	No
West Bay	7	ND	74.3	25.7	2.7	Yes
	8	ND	94.1	5.9	0.3	No
Eel River	9	1.6	28.8	69.6	3.4	No
Cotuit Bay	10	ND	65.6	34.4	1.6	No
	11	ND	81.7	18.3	1.2	No
	12	0.6	95.6	3.8	0.3	No
Prince Cove	13	1.4	27.4	71.2	5.8	Yes
Warren's Cove	15	0.4	91.7	7.9	1.5	No
North Bay	17	ND	56.3	43.7	3.1	Yes
	18	ND	14.4	85.6	4.3	Yes
	19	ND	60.9	39.1	1.0	No
West Bay	22	ND	39.7	60.3	3.3	No
	23	5.2	89.5	5.3	0.2	No
	24	ND	83.3	16.7	0.8	No
	25	ND	13.9	86.1	3.3	Yes
Cotuit Bay	28	ND	20.7	79.3	2.4	No
	29	0.2	88.6	11.2	0.5	No
	30	0.4	51.6	48	1.7	Yes
	31	0.6	31.4	68	2.8	Yes

A few of the sediment grabs recovered macroalgae, but no submerged vascular plants (including eelgrass) were encountered. Laboratory determined infaunal counts ranged from 12 to 1996 individuals per 0.04 m<sup>2</sup> grab, or an extrapolated density of 300 to 49,900 organisms per m<sup>2</sup>. The number of species varied between 2 and 50 per grab, with Shannon-Weiner diversity ranging from 0.1 to 2.3 and Pielou's evenness scores of 0.15-0.83. M-AMBI scores ranged from 0.06-1 (mean: 0.33), or nearly the full spectrum (0-1). See Table 3 for values of each community metric by station and Figure 5 for maps of selected metrics. M-AMBI scores were significantly ( $p < 0.05$ ) and negatively correlated with latitude, water temperature and dissolved oxygen, as well as the amount of fine materials and organic carbon in sediments. M-AMBI scores were significantly and positively correlated with sand content and Secchi depth. Several of these variables were themselves correlated. Pairwise correlation coefficients and their statistical significance are plotted in Figure 6.



**Table 3.** Benthic infaunal community metrics and overall habitat condition. Type F = Fixed, S = Sentinel, R = Randomized. A value of NA for M-AMBI score and habitat condition indicates that the index was not calculated due to overrepresentation (>50%) of individuals in a sample that could not be assigned to an ecological group.

Subembayment	Station	Type	Density (per m <sup>2</sup> )	Number of species (S)	Shannon diversity (H')	Pielou's evenness (E)	M-AMBI score	Habitat condition
Cotuit Bay	10	F	12125	32	1.87	0.54	0.72	good
	11	F	7400	26	2.26	0.69	0.60	good
	12	S	49900	48	2.06	0.53	NA	NA
	28	R	11925	22	1.74	0.56	0.50	moderate
	29	R	10375	41	2.34	0.63	NA	NA
	30	R	300	2	0.56	0.81	NA	NA
	31	R	2025	14	1.71	0.65	0.33	poor
<i>mean</i>			<i>13436</i>	<i>26</i>	<i>1.79</i>	<i>0.63</i>	<i>0.54</i>	<i>good</i>
Eel River	9	F	3500	6	0.38	0.21	0.14	bad
North Bay	4	F	525	3	0.83	0.76	0.10	bad
	5	F	20900	21	1.48	0.49	0.48	moderate
	6	F	1200	2	0.1	0.15	0.06	bad
	17	R	3600	18	1.39	0.48	0.41	moderate
	18	R	625	5	1.33	0.83	0.15	bad
	19	R	2175	8	1.54	0.74	0.22	poor
<i>mean</i>			<i>4838</i>	<i>10</i>	<i>1.11</i>	<i>0.58</i>	<i>0.24</i>	<i>poor</i>
Prince Cove	2	F	850	7	1.29	0.66	0.18	bad
	3	F	2975	7	0.64	0.33	0.17	bad
	13	R	2525	4	0.38	0.28	0.10	bad
<i>mean</i>			<i>2117</i>	<i>6</i>	<i>0.77</i>	<i>0.42</i>	<i>0.15</i>	<i>bad</i>
Warren's Cove	1	F	3100	10	1.6	0.7	0.25	poor
	15	R	950	9	1.46	0.66	NA	NA
<i>mean</i>			<i>2025</i>	<i>9.5</i>	<i>1.53</i>	<i>0.68</i>	<i>0.25</i>	<i>poor</i>
West Bay	7	F	400	6	1.12	0.63	0.17	bad
	8	F	11450	29	1.65	0.49	0.64	good
	22	R	4300	10	1.15	0.5	0.24	poor
	23	R	34775	50	2.11	0.54	1.00	high
	24	R	12100	18	1.73	0.6	0.42	moderate
	25	R	2125	11	1.33	0.56	0.26	poor
<i>mean</i>			<i>10858</i>	<i>21</i>	<i>1.52</i>	<i>0.55</i>	<i>0.46</i>	<i>moderate</i>



**Figure 6.** Pairwise correlations among measured variables. Shallow water column observations are prefixed with “s.”, and deep, or bottom, with “b.”. Units are consistent with those given in Tables 1 & 2. Circle color indicates the value of the Pearson correlation coefficient ( $r$ ). Circle size is proportional to  $|r|$ . Significant relationships are indicated with asterisks according to the following p-value criteria:  $< 0.05$  \*,  $< 0.01$  \*\*,  $< 0.001$  \*\*\*.

M-AMBI scores were assigned to US M-AMBI condition classes (Pelletier et al., 2018) for further assessment. Highly degraded habitat conditions (“poor” or “bad”) were found in all subembayments. Favorable conditions (“good” or “high”) were found only in the most seaward Cotuit and West Bays, where large differences in overall benthic condition were also observed among nearby stations (see Figure 5). Grouped by subembayment, M-AMBI scores were broadly consistent with other observations. Spatially averaged values (see Table 3) correspond with poor or bad habitat condition classes in northern subembayments, and moderate to good condition classes in southern ones. Variability in Cotuit Bay was high enough that its mean M-AMBI score is sensitive to which stations (fixed, randomized, or both) are included; overall habitat condition would be considered good if based on fixed stations, but moderate if based on randomized stations.

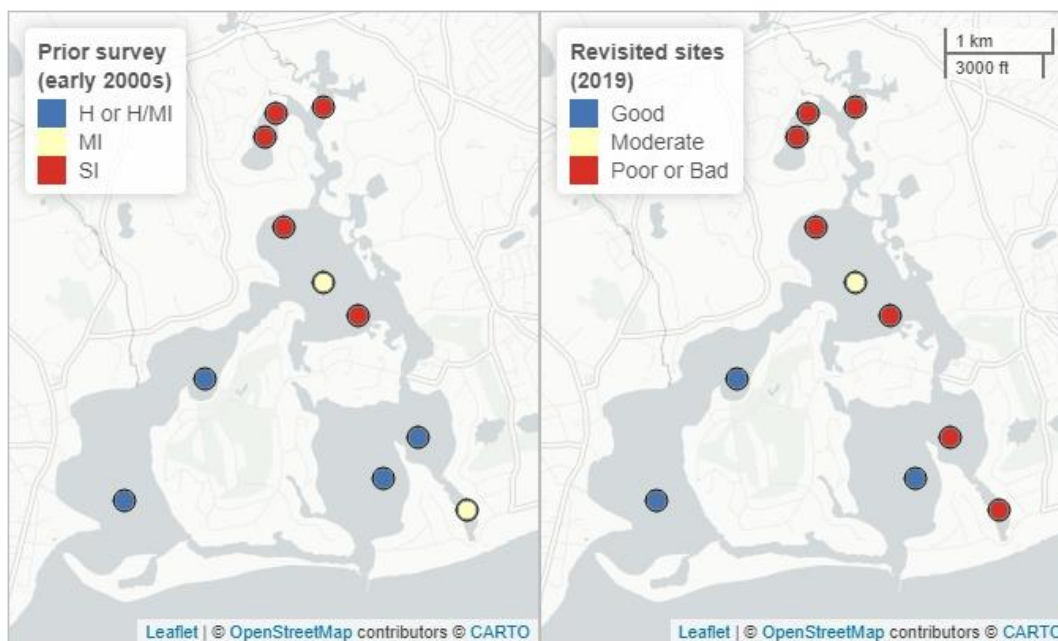
The 2019 infaunal community data suggest that benthic habitat condition has not improved since the prior survey (Howes et al., 2006) and there is some evidence of further degradation. Among the set of revisited, or fixed, stations, the recent survey found a lower density and diversity of organisms, and lower species evenness (Table 4). Note that densities have been extrapolated to a common area (1 m<sup>2</sup>) to facilitate comparison; the prior survey reported data as the average of three grabs per station, each of which sampled a larger surface area (0.0625 m<sup>2</sup>). Changes in the total number of species sampled were less definitive, with a higher mean and lower median among fixed stations compared with the prior survey. Overall change in habitat condition over time was assessed by comparing condition classes used in the past under MEP and those associated with US M-AMBI (see Table 4). Habitat condition shows little to no change at 9 of the 11 fixed stations, while conditions at the remaining 2 have worsened.

**Table 4.** Benthic infaunal community metrics and change in overall habitat condition over time. *S* = number of species, *H'* = Shannon diversity, *E* = Pielou’s evenness. Change in overall condition was assessed by making equivalences between the condition classes used in the prior MEP survey (“Significant Impairment (SI)”, “Moderate Impairment (MI)”, “Healthy habitat (H)”), and the respective groupings of M-AMBI classes (“Bad/Poor”, “Moderate”, “Good/High”).

Station & subembayment	prior MEP survey (early 2000s)					current survey (2019)						Change in overall condition
	Density (per m <sup>2</sup> )	<i>S</i>	<i>H'</i>	<i>E</i>	Infaunal Indicators	Density (per m <sup>2</sup> )	<i>S</i>	<i>H'</i>	<i>E</i>	M-AMBI score	Habitat condition	
1. Warren’s Cove	112	4.7	2.01	0.86	SI	3100	10	1.60	0.70	0.25	poor	no change
2. Prince Cove	800	9.3	2.49	0.82	SI	850	7	1.29	0.66	0.18	bad	no change
3. Prince Cove	688	4.7	0.9	0.43	SI	2975	7	0.64	0.33	0.17	bad	no change
4. North Bay	176	4.7	1.9	0.85	SI	525	3	0.83	0.76	0.11	bad	no change
5. North Bay	13136	14.3	1.35	0.36	MI	20900	21	1.48	0.49	0.48	moderate	no change
6. North Bay	112	3	1.91	0.92	SI	1200	2	0.10	0.15	0.06	bad	no change
7. West Bay	8016	17.3	3.39	0.82	H/MI	400	6	1.12	0.63	0.18	bad	<b>worse</b>
8. West Bay	30320	26.3	2.02	0.42	H/MI	11450	29	1.65	0.49	0.65	good	no change
9. Eel River	7456	11	2.28	0.67	MI	3500	6	0.38	0.21	0.14	bad	<b>worse</b>
10. Cotuit Bay	8560	16.3	2.99	0.75	H/MI	12125	32	1.87	0.54	0.71	good	no change
11. Cotuit Bay	3728	16	3.26	0.82	H	7400	26	2.26	0.69	0.60	good	no change
<i>mean</i>	<i>6646</i>	<i>12</i>	<i>2.23</i>	<i>0.70</i>		<i>5857</i>	<i>14</i>	<i>1.20</i>	<i>0.51</i>	<i>0.32</i>	<i>poor</i>	
<i>median</i>	<i>3728</i>	<i>11</i>	<i>2.02</i>	<i>0.82</i>		<i>3100</i>	<i>7</i>	<i>1.29</i>	<i>0.54</i>	<i>0.18</i>	<i>bad</i>	

The longitudinal comparison of habitat condition is less than ideal for several reasons. The dates of the prior benthic survey and coordinates of the surveyed locations were not published. Here fixed station locations were georeferenced from the published map (Howes et al., 2006). There are some differences in sampling methods (discussed above) and assessment methodology. The

summary ratings for habitat condition used in the prior survey (see “Infaunal Indicators” in Table 4) were not described in enough quantitative detail to apply to the current one. The correspondence over time in overall assessed condition at most stations, however, suggests a signal that is stronger than these sources of noise. The probability of finding no overall improvement in benthic habitat at any of the fixed stations is less than 1% if 7 of the 11 sites could have shown improvement due to random chance. Conversely, 6 of the revisited sites had the potential to get worse, and 2 did (see Figure 7), indicating that the classification is at least subject to change. The direction of change (worse) is consistent with expectations, given the limited scope of contaminant mitigation measures. The relative influence of nutrient loading versus other factors, like climate, on changes in benthic condition in Three Bays has not been evaluated here.



**Figure 7.** Comparison of habitat condition assessments from prior and current benthic surveys. Colors reflect equivalences that were made between the condition classes used in the prior MEP survey (“Significant Impairment (SI)”, “Moderate Impairment (MI)”, “Healthy habitat (H)”), and the respective groupings of US M-AMBI classes (“Bad/Poor”, “Moderate”, “Good/High”). None of the revisited sites had “High” quality benthic habitat condition in the 2019 survey, though one of the randomized stations did (see Figure 5).

The current survey also included locations that were not previously assessed, yielding a more nuanced perspective on current conditions. The TMDL sentinel station has regulatory significance; its location was chosen such that meeting water quality goals there would indicate an expectation of goal attainment throughout the Three Bays system (MassDEP, 2007).

Interestingly, the sentinel had the highest density of organisms and second highest number of species of any station. An overabundance of organisms that could not be assigned to an ecological group precluded determination of the M-AMBI score and condition class at the sentinel and three of the randomized stations. The latter were added to the 2019 survey to avoid selection biases that may be present in the fixed set of stations. Randomized stations had a higher average density, species count, diversity, and M-AMBI score than fixed stations. M-AMBI scores for randomized stations also showed greater spatial variability (see Figure 5), as was previously discussed. Both fixed stations in Cotuit Bay were classified as good, for example, whereas two nearby randomized locations were assessed to be in moderate to poor condition.

Use of an established benthic index supports analysis of habitat condition in Three Bays as well as comparison with other estuaries. The M-AMBI score provides a quantitative summary of infaunal community data that can be used to standardize assessment. Widely used around the world (see, e.g., Sigovini et al., 2013), M-AMBI is also currently being applied to other estuaries on Cape Cod as part of a MEP review of benthic monitoring procedures. In nearby West Falmouth Harbor, a recent benthic survey applied M-AMBI and showed that habitat conditions had remained largely unchanged over a 16-year period at some of the revisited stations (Sweeny and Rutecki, 2020). Other stations showed improvements, including re-established eelgrass beds, that may be related to upgraded nutrient control at a nearby wastewater treatment plant. Pending results for Pleasant Bay and Wellfleet Harbor may be considered alongside these to learn how estuarine habitats across Cape Cod respond to differences in nutrient loading and interventions to manage it.

### *Acknowledgments*

The authors appreciate assistance with planning and executing the 2019 benthic survey provided by Marty Chintala and Zee Crocker, guidance on the application of M-AMBI provided by Peg Pelletier and Giancarlo Cicchetti. They are grateful for comments from internal reviewers that have improved this data summary. The work was funded by the US EPA Office of Research and Development's (ORD) Safe and Sustainable Water Resources program. It is identified by ORD-041256.

### *Disclaimer*

This document and the associated supplemental materials include contributions from The U.S. Environmental Protection Agency (EPA) and individuals outside the United States Government. EPA, through its Office of Research and Development, funded this effort and managed the contracted analyses described herein. This data summary has been subjected to the Agency's review and has been approved for publication. Mention of trade names, products, or services does not convey EPA approval, endorsement, or recommendation. The views expressed in this summary are those of the author(s) and do not necessarily represent the views or the policies of the U.S. Environmental Protection Agency.

## References

- Batiuk R. A., Orth R. J., Moore K. A., Dennison W. C., Stevenson J. C., Staver L. W., Carter V., Rybicki N. B., Hickman R. E., Kollar S., Bieber S., Heasley P. 1992. Chesapeake Bay submerged aquatic vegetation habitat requirements and restoration targets: A technical synthesis. US Environmental Protection Agency, Annapolis, MD.
- Borja A., Franco J., Pérez, V. 2000. A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. *Mar. Pollut. Bull.* 40, 1100–1114.
- Gillett D.J., Weisberg S. B., Grayson T., Hamilton A., Hansen V., Leppo E. W., Pelletier M. C., Borja A., Cadien D., Dauer D., Diaz R., Dutch M., Hyland J. L., Kellogg M., Larsen P. F., Levinton J. S., Llansó R., Lovell L. L., Montagna P. A., Pasko D., Phillips C. A., Rakocinski C., Ranasinghe J. A., Sanger D. M., Teixeira H., VanDolah R. F., Velarde R. G., Welch K. I. 2015. Effect of ecological group classification schemes on performance of the AMBI benthic index in US coastal waters. *Ecol. Indic.* 50, 99–107.
- Howes B., Kelley S.W., Ramsey J. S., Samimy R., Schlezinger D., Eichner E. 2006. Linked Watershed-Embayment Model to Determine Critical Nitrogen Loading Thresholds for Three Bays, Barnstable, Massachusetts. Massachusetts Estuaries Project, Massachusetts Department of Environmental Protection. Boston, MA.
- Hyland J., Balthis L., Karakassis I., Magni P., Petrov A., Shine J., Vestergaard O., Warwick R. 2005. Organic carbon content of sediments as an indicator of stress in marine benthos. *Mar. Ecol. Prog Ser.* 295: 91-103.
- Kelley D. and Richards C. 2020. *oce*: Analysis of Oceanographic Data. R package version 1.2-0.
- Kincaid T. M., Olsen, A. R. 2019, Weber M. H. *spsurvey*: Spatial Survey Design and Analysis. R package version 4.1.0.
- MassDEP. 2007. Three Bays System Total Maximum Daily Loads for Total Nitrogen. Massachusetts Department of Environmental Protection. Report # 96-TMDL-10 Control # 242.0.
- Muxika I., Borja Á., Bald J. 2007. Using historical data, expert judgement and multivariate analysis in assessing reference conditions and benthic ecological status, according to the European water framework directive. *Mar. Poll. Bull.* 55, 16–29.
- Oksanen J., Blanchet., F.G., Friendly M., Kindt R., Legendre P., McGlenn D., Minchin P.R., O'Hara R. B., Simpson G. L., Solymos P., Henry M., Stevens H., Szoecs E., Wagner H. 2019. *vegan*: Community Ecology Package. R package version 2.5-6.
- Pelletier M. C., Campbell D. E., Ho K. T., Burgess R. M., Audette C. T., Detenbeck N. E. 2011. Can sediment total organic carbon and grain size be used to diagnose organic enrichment in estuaries? *Environ. Toxicol. Chem.* 30(3), 538-547.



Pelletier M. C., Gillett D. J., Hamilton A., Grayson T., Hansen V., Leppo E. W., Weisberg S. B., Borja A. 2018. Adaptation and application of multivariate AMBI (M-AMBI) in US coastal waters. *Ecol. Indic.* 89, 818-827.

Sigovini M., Keppel E., Tagliapietra D. 2013. M-AMBI revisited: looking inside a widely-used benthic index. *Hydrobiologia* 717, 41–51.

Stevens D. L., Olsen A. R. 2004. Spatially Balanced Sampling of Natural Resources, *J. Am. Stat. Assoc.*, 99:465, 262-278, DOI: 10.1198/016214504000000250

Sweeny M., Rutecki D. A. 2020. Benthic monitoring report: West Falmouth Harbor 2019. Prepared for the MassDEP Massachusetts Estuaries Project. June 2020. 49pp.

US EPA. (U.S. Environmental Protection Agency). 1995. Environmental Monitoring and Assessment Program (EMAP): Laboratory Methods Manual - Estuaries, Volume 1: Biological and Physical Analyses. United States Environmental Protection Agency, Office of Research and Development, Narragansett, RI. EPA/620/R-95/008.

US EPA (U.S. Environmental Protection Agency). 2015. National Coastal Condition Assessment: Field Operations Manual. Version 1.0, May 2015. EPA-841-R-14-007. U.S. Environmental Protection Agency, Office of Water, Washington, DC. 166 pp.