 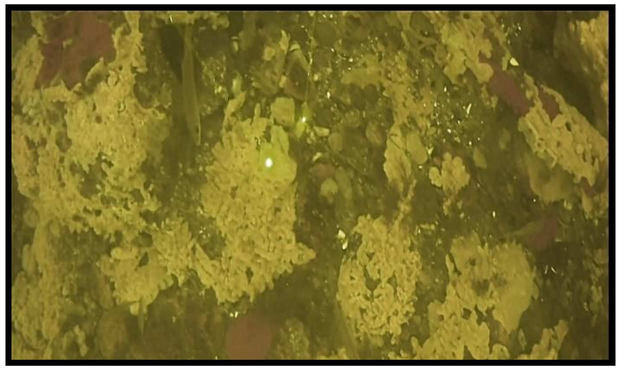

Multi-Community Partnership to Reduce Nitrogen in Upper Buzzards Bay – Benthic Analysis

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1. Introduction

1.1 Overview

Communities throughout southeastern Massachusetts and Cape Cod depend on good water quality to support their local economies and quality of life. However, many are struggling with how to reduce nitrogen entering harbors and coves in an effective, affordable, and sustainable way. The Multi-Community Partnership to Reduce Nitrogen in Upper Buzzards Bay Project ("Project") capitalizes on the collective need to solve the nitrogen pollution problem in two nitrogen-impaired upper Buzzards Bay estuaries, namely, the Agawam/Wareham River and Buttermilk/Little Buttermilk Bay. In addition, the Project will meet the economic development needs in the town of Bourne and save a state educational institution from investing in costly upgrades to existing wastewater infrastructure.

This Project is a partnership between the Buzzards Bay Coalition (BBC), the Massachusetts Maritime Academy (MMA), and the Towns of Wareham, Bourne, and Plymouth. Bringing together all of the "Head of the Bay" communities, in addition to MMA and BBC, to combine resources and municipal sewer infrastructure across town and watershed boundaries will result in a regional wastewater solution to reduce nitrogen pollution in the two aforementioned sub-estuaries. This will be achieved by relocating the Town of Wareham's highly-performing wastewater discharge outfall from the Agawam/Wareham River, a nitrogen-impaired waterbody, to Cape Cod Canal and expanding the municipal sewer system in the region.

1.2 Objective

Until recently, Massachusetts state law prohibited the permitting of new municipal wastewater ocean discharges in state-designated ocean sanctuaries, including Buzzards Bay. The passage of Chapter 259 of the Acts of 2014 allows Massachusetts to approve ocean discharges in ocean sanctuaries when robust scientific evidence shows that there is no adverse impact to ocean water quality or groundwater quality, and the new discharge receives advanced treatment for nitrogen. The findings from a previously funded 2015 Southeast New England Project (SNEP), The Multi-Community Partnership to Reduce Nitrogen to Buzzards Bay, concluded that relocating the Town of Wareham's wastewater treatment discharge pipe from the Agawam/Wareham River to Cape Cod Canal with an expanded discharge was feasible, would not adversely affect the canal's water quality, and could result in an approximately 90,000 pound reduction in nitrogen per year.

This Project continues the partnership and proceeds with the next critical steps towards assessing feasibility. As part of this Project, benthic surveys were performed to assess the current habitat quality in the vicinity of the proposed discharge location in Cape Cod Canal. At the bottom of the water column, the area around the seafloor – or benthic environment – collects particles that sink including dead plankton, fecal pellets of marine organisms, and sediment. The benthic environment, thus collects material from the water column over time, providing an indication of the ecosystem status of the waters above. The sediment characteristics (e.g., percent organic material) and the organisms present in the sediments provide valuable information about overall ecosystem health.

2. Methods

Five sampling stations were identified after consulting with officials from Massachusetts Department of Environmental Protection (MassDEP), Massachusetts Office of Coastal Zone Management, Massachusetts Division of Marine Fisheries, and scientific experts from the MMA, the Marine Biological Laboratory, the Woods Hole Oceanographic Institution, and the Woods Hole Research Center (Figure 1). The monitoring stations were selected to evaluate the habitat closest to the proposed outfall location, as well as sensitive eelgrass beds in the vicinity. The station IDs' latitude and longitude can be found in Table 1.

Figure 1: Benthic Survey Stations (orange triangles) and existing MMA outfall (red circle).

The benthic survey was completed on three separate days, 10/5/18, 10/10/18, and 10/26/18, due to the volume of traffic and significant currents in the Cape Cod Canal. All stations, except MMA5, were accessed by a 28-foot research vessel provided by MMA. Prior to benthic sample collection, approximately two minutes of video footage and at least five still photos were taken of the bottom at each station. In addition, a multi-parameter sonde was used to measure basic water quality parameters (temperature, dissolved oxygen, salinity, pH). Water quality measurements were taken at 0.15m, 0.5m, every 1m from 1-10m, and every 5m thereafter. Four replicate grabs were then collected using a $0.04m^2$ Van Veen grab for benthic macrofauna analysis, and an additional grab for sediment grain size and total organic carbon (TOC) analysis.

Table 1: Benthic Survey Stations' GPS Coordinates

Station ID	Latitude (°N)	Longitude (°W)
MMA7	41.74235	-70.6152
MMA6	41.73993	-70.6221
MMA5	41.73866	-70.6268
MMA4	41.72905	-70.6312
MMA3	41.73682	-70.6287

Of the four replicate grabs collected, three were processed for benthic macrofauna identification with the remaining one archived for potential future reference. Total organic carbon samples were analyzed by Alpha Analytical using the Lloyd Kahn method, and sediment grain size analysis was completed by BBC and MMA personnel at MMA's Aquaculture Lab using standard tested nesting sieves.

Lastly, because MMA6 and MMA7 were located in mussel beds, the length of a subset of the mussels collected in each sample was measured to establish baseline conditions and to monitor mussel length over time. The Northeast Fisheries Science Center (NEFSC 2016) recommends subsampling at least 20% of the catch. Therefore, 20% of the total mussels in each grab were measured, but no fewer than 30 individuals. To select the subset, mussels from each sample were arranged from smallest to largest and numbered consecutively. A list of numbers was created using a random number generating tool and the corresponding mussels were then measured.

A thorough description of sampling methods can be found in the BBC's Quality Assurance Project Plan/Embayment Specific Study Plan for Multi-Community Partnership to Reduce Nitrogen in Upper Buzzards Bay – Benthic Analysis (Jakuba and Hubbard, 2018) and MassDEP's September 2018 Massachusetts Estuaries Project Benthic Monitoring Quality Assurance Project Plan (QAPP) Draft Version 1 (Rutecki and Nestler, 2018). The only deviation from the QAPPs involved the sediment grain size and TOC analysis. Because MMA6 and MMA7 were located in mussel beds, an insufficient amount of sediment was collected to perform these analyses. Therefore, sediment grain size and TOC analysis was completed for only MMA3, MMA4, and MMA5.

3. Results

The benthic survey revealed four distinct benthic communities within Upper Buzzards Bay; a *Mytilus edulis* bed, a *Zostera marina* L. community, a sand flat with coarse sand bottom, and a *Crepidula fornicata* community. MMA6 and MMA7, located in the previously dredged, fast-current Cape Cod Canal, consist predominately of *Mytilus edulis* and large boulders. MMA4, located within MassDEP mapped eelgrass, revealed eelgrass and coarse and medium sand bottoms, whereas MMA3, also located within MassDEP mapped eelgrass beds, didn't reveal eelgrass but a coarse sand bottom. Lastly, MMA5 was located in a *Crepidula fornicata* community where the highest number of species and individual organisms were present.

3.1 Photo/video

Video footage and still photos were taken at each station prior to collecting benthic samples. Below are selected pictures of each station's bottom substrate with a url to the video footage. Two laser points are shown in each frame as a 10cm scale reference. Additional pictures can be found in Appendix I.

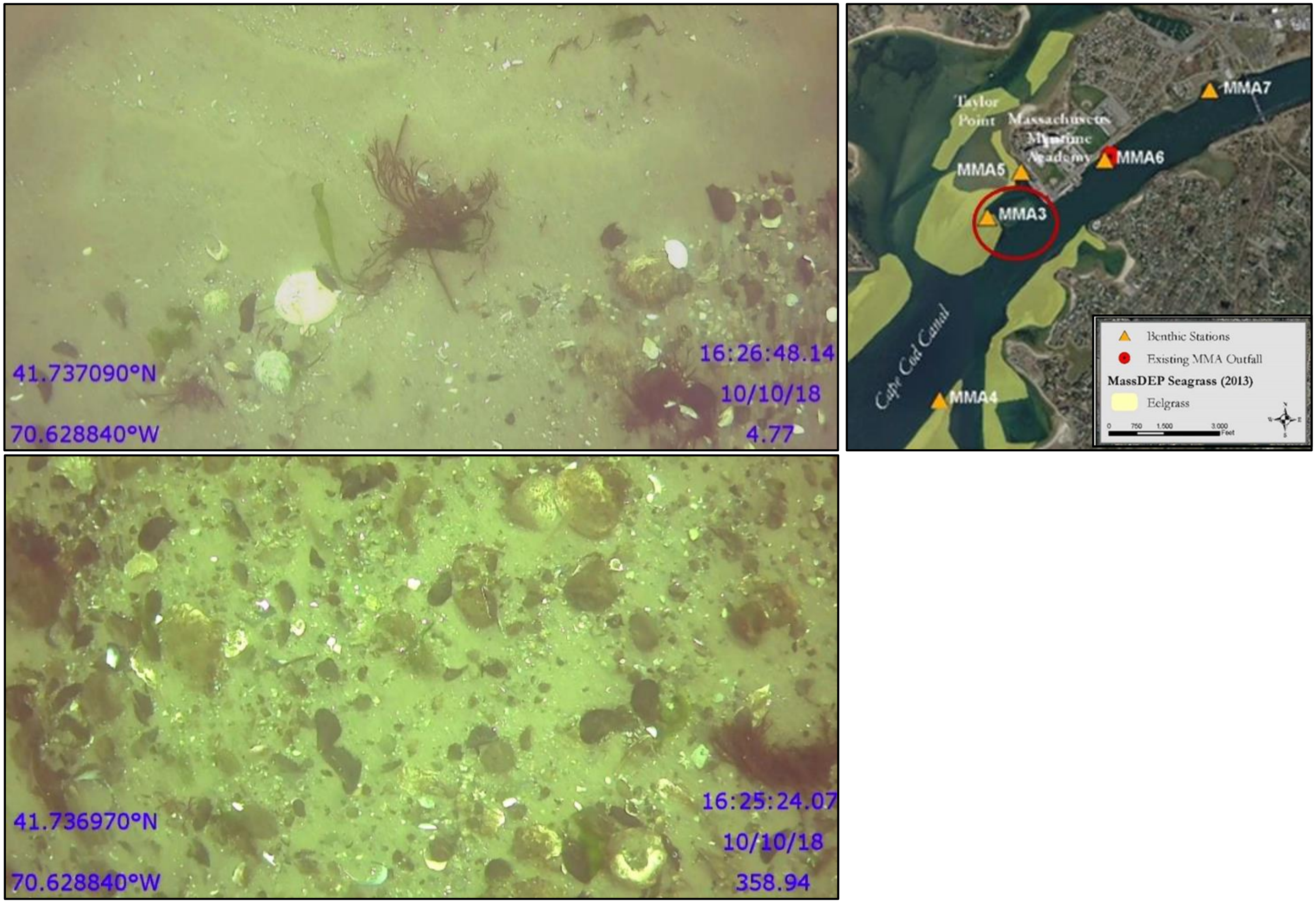

Figure 2: Station MMA3 is within a DEP mapped eelgrass bed. Still photos depict a coarse sandy bottom with some shells/shell fragments. Some sea lettuce (*Ulva*) present with higher abundances of the green seaweed commonly referred to as dead man's fingers (*Codium*). Link to video footage here: https://www.youtube.com/watch?v=Kw8IhYVNpbA&feature=youtu.be

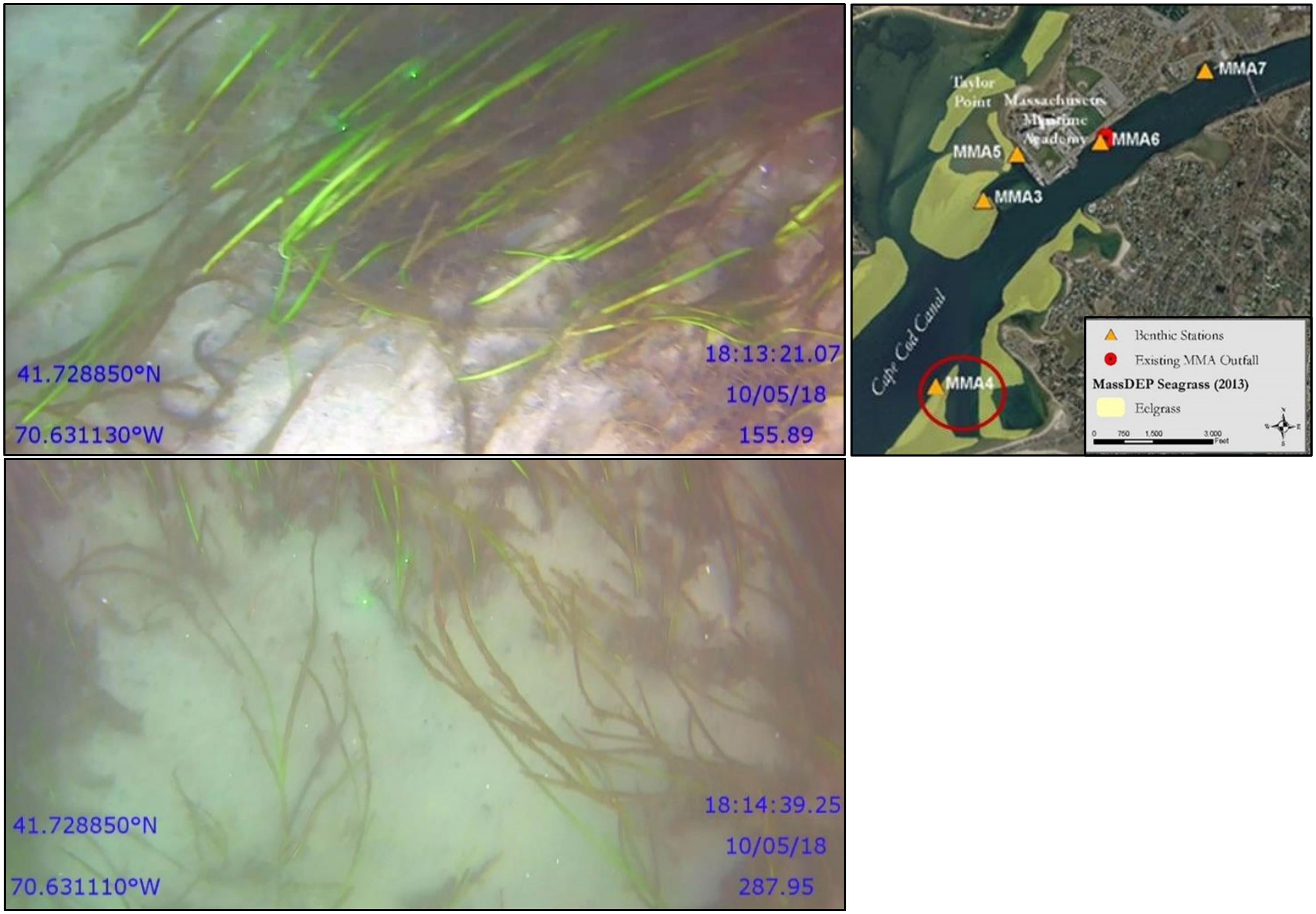

Figure 3: Station MMA4 is at the edge of a DEP mapped eelgrass bed. Still photos depict a medium sandy bottom with stalks of eelgrass, some of which have epiphytes. Link to video footage here: https://www.youtube.com/watch?v=khoR9i_QRSk&feature=youtu.be

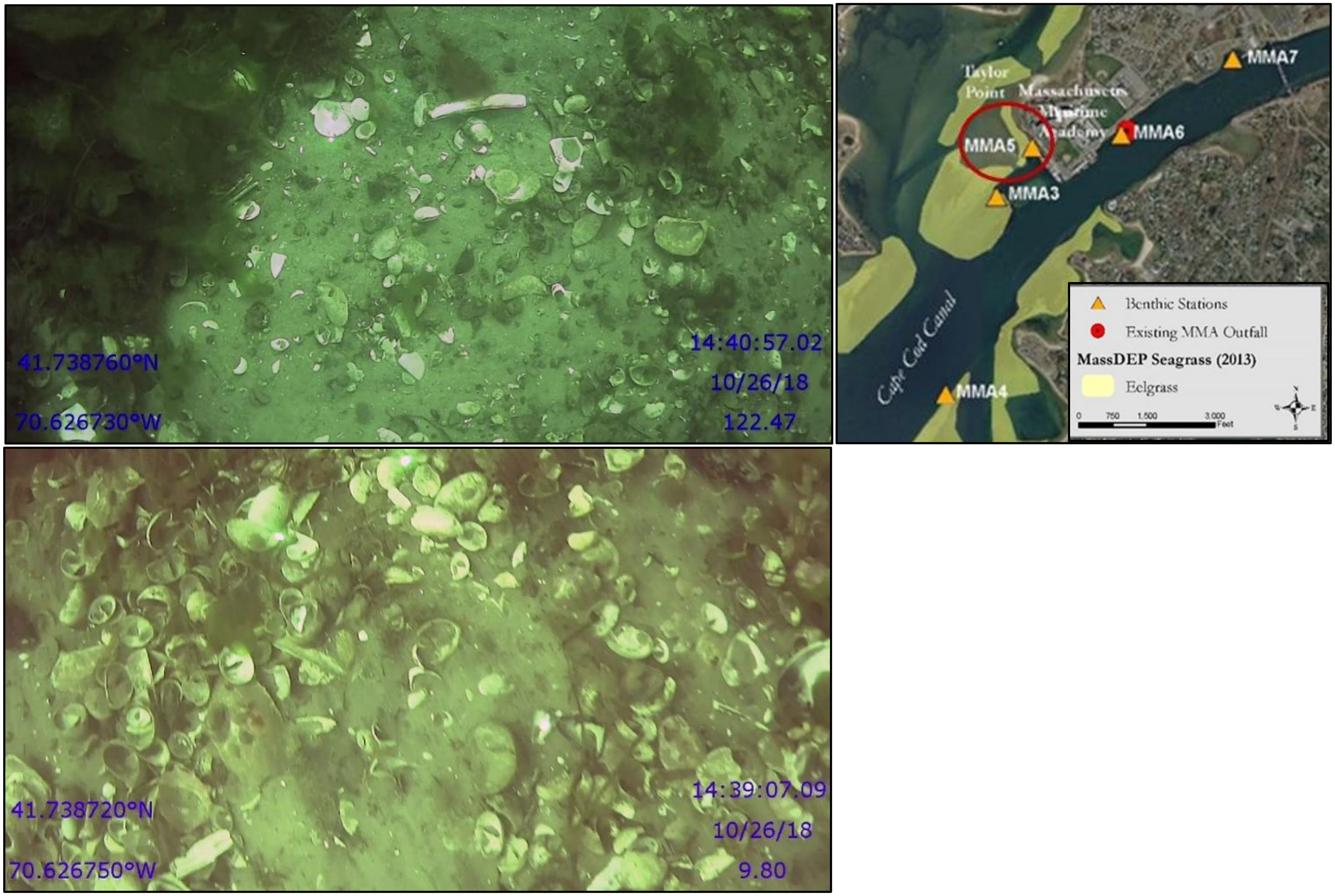

Figure 4: Station MMA5 is within a *Crepidula fornicata* bed. Still photos depict coarse sandy bottom with numerous *Crepidula fornicata* and other mollusks. Brown (*Phaeophyceae*) and green (*Chlorophyta*) algae are also present and dense in some areas. Link to video footage here: https://www.youtube.com/watch?v=OOzAXPyTVL4&feature=youtu.be

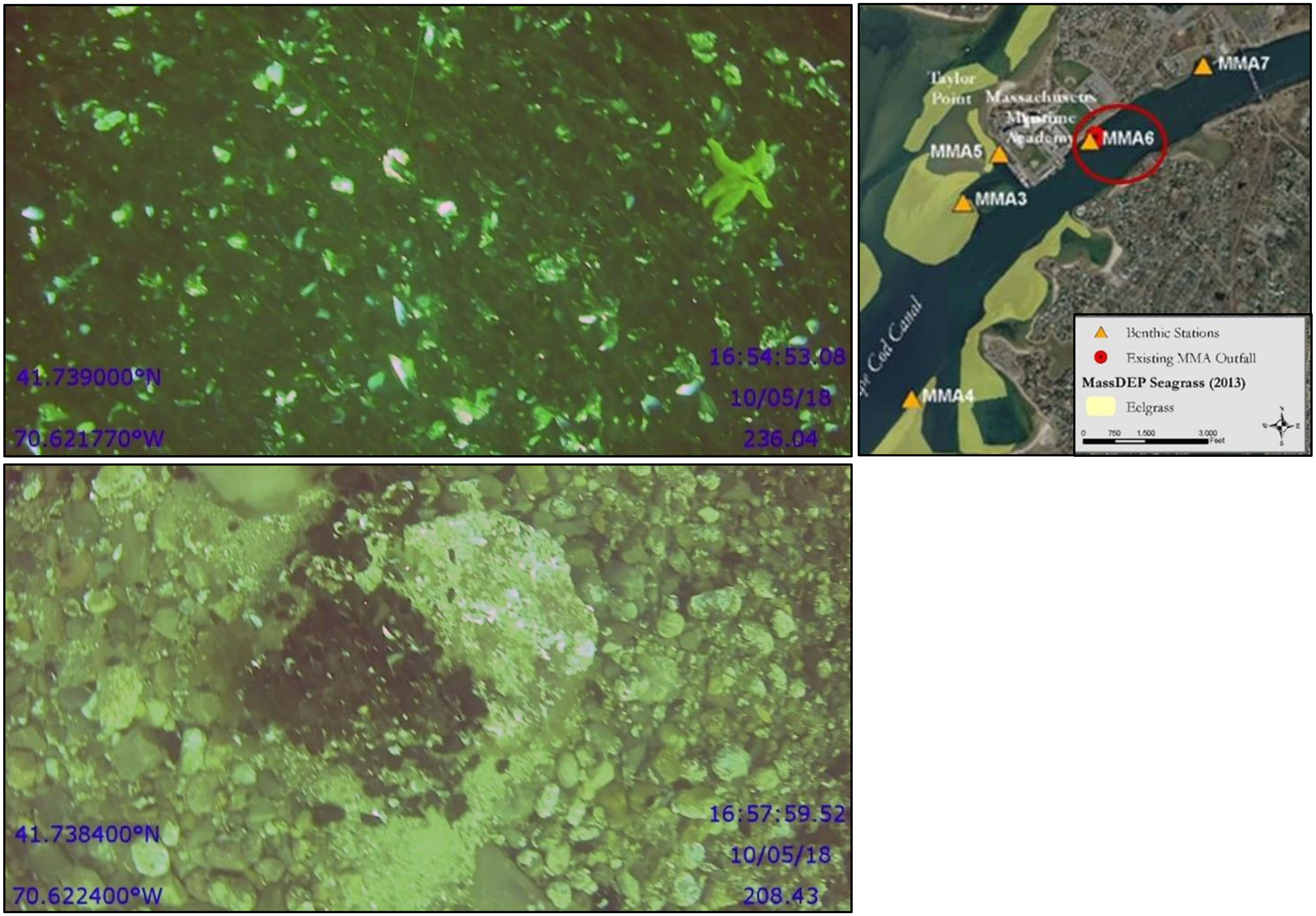

Figure 5: Station MMA6 is within the Cape Cod Canal, a high current area that was previously dredged, in front of a wastewater discharge outfall. Still photos depict a widespread mussel bed (*Mytilus edulis*) with cobble. Link to video footage here: https://www.youtube.com/watch?v=qIGcYluEGjs&feature=youtu.be

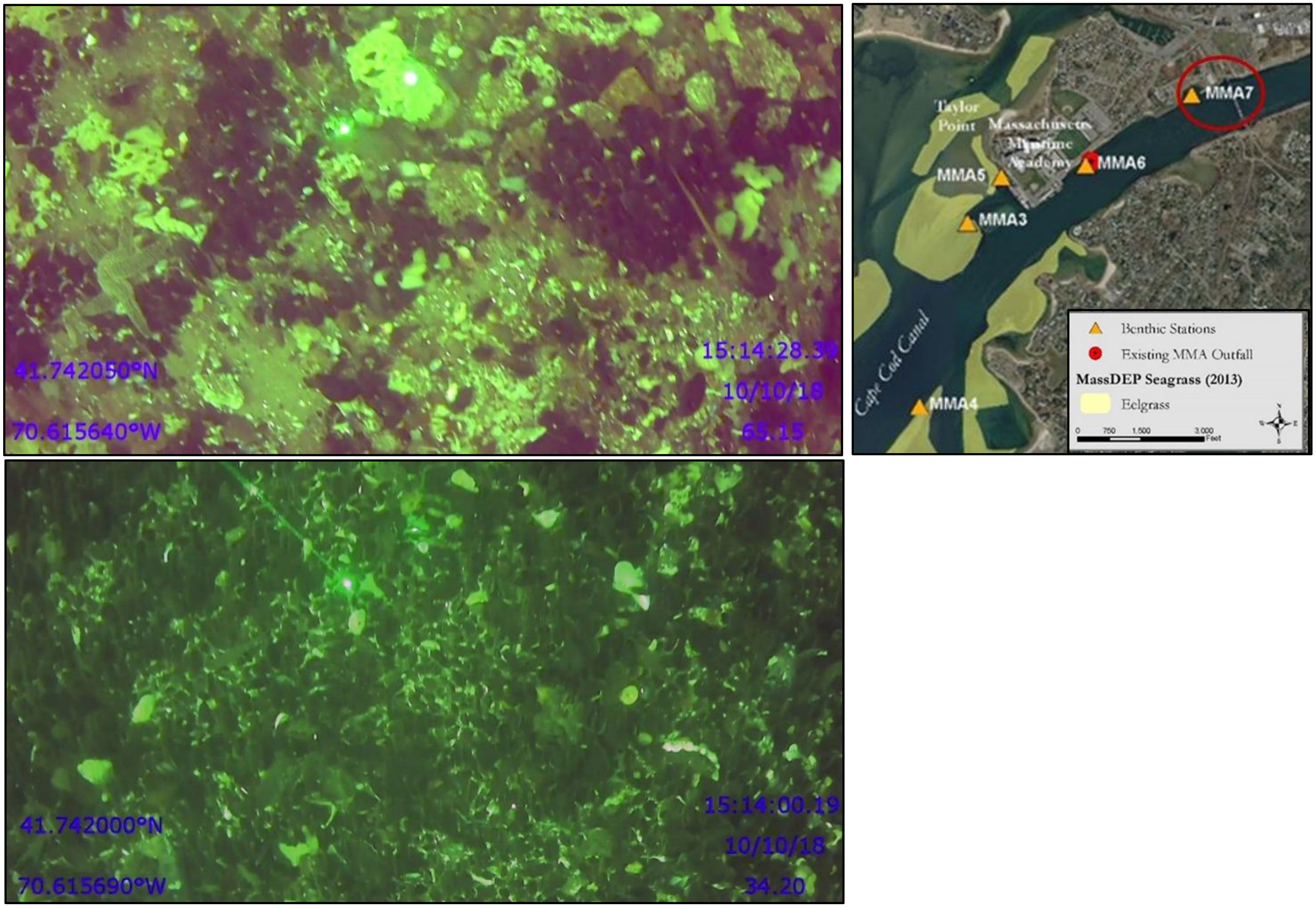

Figure 6: Station MMA7 is within the Cape Cod Canal, a high current area that was previously dredged, northeast of a wastewater discharge outfall. Still photos depict a widespread mussel bed (*Mytilus edulis*) with many boulders and cobble. Link to video footage here: https://www.youtube.com/watch?v=0Zbtpb6zFDQ&feature=youtu.be

3.2 Water Quality Measurements

Basic water quality parameters (temperature, dissolved oxygen, pH, and salinity) were recorded at various depths prior to grabbing benthic samples as described in MassDEP's Massachusetts Estuaries Project Benthic Monitoring QAPP – section A7.1.2 (Rutecki and Nestler, 2018). Table 2 summarizes water quality measurements at each station 0.15m below the surface and within 1m from the bottom. All water quality measurements for each station can be found in Appendix II.

Table 2: Surface and Deep Water Quality Measurements at Each Station (EB=Eelgrass Bed, CFB=*Crepidula fornicata* bed, CCC=Cape Cod Canal).

Station	Sampling Date	Total Depth (m)	Sample Depth (m)	Temperature (°C)	Salinity	Dissolved Oxygen (mg/L)	Dissolved Oxygen (%)	pH
MMA3	10/10/18	5.0						
Surface			0.15	18.7	31.24	7.50	96.4	7.84
Deep			4.79	18.7	31.21	7.70	99.0	7.83
MMA4 (EB)	10/5/18	3.0						
Surface			0.15	18.0	31.58	7.86	99.6	8.35
Deep			2.94	18.1	31.57	7.81	99.0	8.33
MMA5 (CFB)	10/26/18	9.0						
Surface			0.15	11.6	29.40	8.70	95.6	7.79
Deep			8.76	11.6	29.40	8.67	95.3	7.78
MMA6 (CCC)	10/5/18	8.5						
Surface			0.15	17.7	31.70	7.69	96.7	8.04
Deep			7.89	17.7	31.69	7.44	93.6	8.20
MMA7 (CCC)	10/10/18	13.0						
Surface			0.15	18.5	31.23	7.39	94.5	7.80
Deep			12.85	18.5	31.21	7.28	93.1	7.79

3.3 Benthic Collection and Macrofauna Analysis

Four replicate grabs were collected at each station. Three grabs were processed for benthic macrofauna identification and one was archived for potential future reference. The sample collection and processing procedures used are described in the MassDEP's Massachusetts Estuaries Project Benthic Monitoring QAPP – section B2.2 and B4.1 (Rutecki and Nestler, 2018) and the Embayment Specific Plan within BBC's QAPP (Jakuba and Hubbard, 2018). Organisms were identified to species level and counted in each sample as described in section B4.1 of MassDEP's Massachusetts Estuaries Project Benthic Monitoring QAPP. Sample residues are being retained until this report is accepted by MassDEP and reference collection specimens are being retained until the next survey (3 to 5 years) as outlined in the MassDEP Massachusetts Estuaries Project Benthic Monitoring QAPP – 3.2.3.

The average number of total species at each station ranged from 16 to 42 (Table 3). The variation between replicates of the same station was lowest at the two Cape Cod Canal stations (6-10%) that were in mussel beds. Higher variation (17-36%) was observed between replicates of the stations located in eelgrass or *Crepidula fornicata* beds. The average number of total species was lowest at MMA6 and MMA7 in the Cape Cod Canal. The highest number of species were observed at MMA4 (eelgrass bed) and MMA5 (*Crepidula fornicata* bed). MMA3 (eelgrass bed) had an intermediate number of average total species.

Table 3: Summary of the number of species and individuals present, with accompanying standard deviation, and the most abundant species at each station.

Station	Total Species	Total Individuals	Most Abundant	2nd Most Abundant	3rd Most Abundant
MMA3	25±9	253±167	*Microphthalmus aberrans*	*Parapionosyllis longcirrata*	*Leitoscoloplos fragilis*
MMA4	35±7	327±139	*Salvatoria clavata*	*Mediomastus ambiseta*	*Dodecaceria coralii*
MMA5	42±7	797±218	*Mediomastus ambiseta*	*Lembos websteri*	*Astyris lunata*
MMA6	16±1	174±75	*Mytilus edulis*	*Idotea balthica*	*Astyris lunata*
MMA7	20±2	504±118	*Mytilus edulis*	*Idotea balthica*	*Astyris lunata*

The average total number of individuals at each station ranged from 174 to 797 (Table 3). The variation in the number of total individuals between replicates of a single station ranged from 23 to 66%. The highest number of organisms were observed at MMA5.

A complete list of identified species in each sample can be found in Appendix III. Table 3 provides a summary of the most abundant species at each station. The most abundant species at MMA6 and MMA7 was *Mytilus edulis*, the blue mussel. The most abundant species at MMA3, MMA4, and MMA5 was an annelid: *Microphthalmus aberrans*, *Salvatoria clavata*, and *Mediomastus ambiseta*, respectively.

Five diversity indices were calculated using PRIMER 6 software for each replicate and then averaged to represent the station. Table 4 provides a summary of the diversity indices (Margalef's Species Richness, Pielou's Evenness Index, Fisher's Alpha Index, Shannon-Weiner Index, Simpson Index). All five diversity indices had higher values at stations MMA3, MMA4, and MMA5 than at stations MMA6 and MMA7.

Table 4: Calculated average diversity indices for all stations with accompanying standard deviation.

Station	Margalef's Species Richness (d)	Pielou's Evenness Index (J´)	Fisher's Alpha Index (α)	Shannon-Weiner Index (H´)	Simpson Index (1 - λ)
MMA3	4.76±1.92	0.715±0.216	10.05±7.27	2.32±0.88	0.805±0.204
MMA4	5.99±1.09	0.768±0.040	10.43±2.69	2.73±0.10	0.900±0.012
MMA5	6.12±1.14	0.631±0.050	9.51±2.37	2.35±0.25	0.808±0.059
MMA6	3.03±0.45	0.499±0.121	4.65±1.07	1.40±0.37	0.531±0.138
MMA7	3.07±0.39	0.524±0.152	4.21±0.70	1.58±0.50	0.623±0.193

3.4 Sediment Grain Size Analysis

An additional benthic grab was collected at each station and split for sediment grain size analysis and total organic carbon analysis. The portion of the grab collected for sediment grain size analysis was dried and then processed through nested sieves (4mm, 2mm, 0.5mm, 0.25mm, 0.125mm, and 0.063mm) to determine the percent by weight of particle size using Coastal and Marine Ecological Classification Standard (CMECS) sediment grain size descriptors. Sediment grain size analysis could not be completed at MMA6 and MMA7 because an insufficient amount of sediment was retrieved due to the abundance of *Mytilus edulis* and cobble.

At stations MMA3 and MMA4, the size fractions characterizing the greatest amount of weight were those representative of coarse (0.5 to <2mm) and medium (0.25 to <0.5mm) sand (Table 5). The size fraction that had the highest weight at station MMA5 was the largest fraction (≥4mm), which is typically classified as large pebbles and sticks. In this case, the largest fraction contained shell/shell fragments which were predominately *Crepidula fornicata* shell. At all three stations, the smallest size fraction (<0.063mm) had the least weight of any of the size fractions.

Table 5: Sediment grain size analysis by weight (g) for stations MMA3, MMA4, and MMA5

Station	Large Pebbles/Sticks (≥4mm)	Gravel (2 to <4mm)	Coarse Sand (0.5 to <2mm)	Medium Sand (0.25 to <0.5mm)	Fine Sand (0.125 to <0.25mm)	Very Fine Sand (0.063 to <0.125mm)	Silt (<0.063mm)
MMA3	37.4	33.6	271.7	93.6	2.7	0.1	0.1
MMA4	98.3	37.9	347.2	273.4	70.9	8.5	1.5
MMA5	147.0	26.3	42.3	109.1	62.8	24.2	11.1

Figure 7 shows the relative percentage of each size fraction for each sample. At MMA3 and MMA4, the dominant size fraction was coarse sand at 62% and 42%, respectively (Figure 7). Medium sand was the second highest percentage at both sites at 21% and 33%, and the 3rd most abundant class of sediments was the largest size fraction at both sites (Figure 7). MMA4 had a larger percentage of fine sand (8%) than MMA3 (1%), whereas MMA4 had a smaller percentage of gravel (4%) than MMA3 (8%).

At MMA5, located in the *Crepidula fornicata* bed, the biggest size fraction represented the largest percentage of sediments (35%), with medium sand (26%) and fine sand (15%) as the next two most abundant size fractions. Coarse sand (10%), gravel (6%), very fine sand (6%), and silt (2%) made up smaller fractions of the sample.

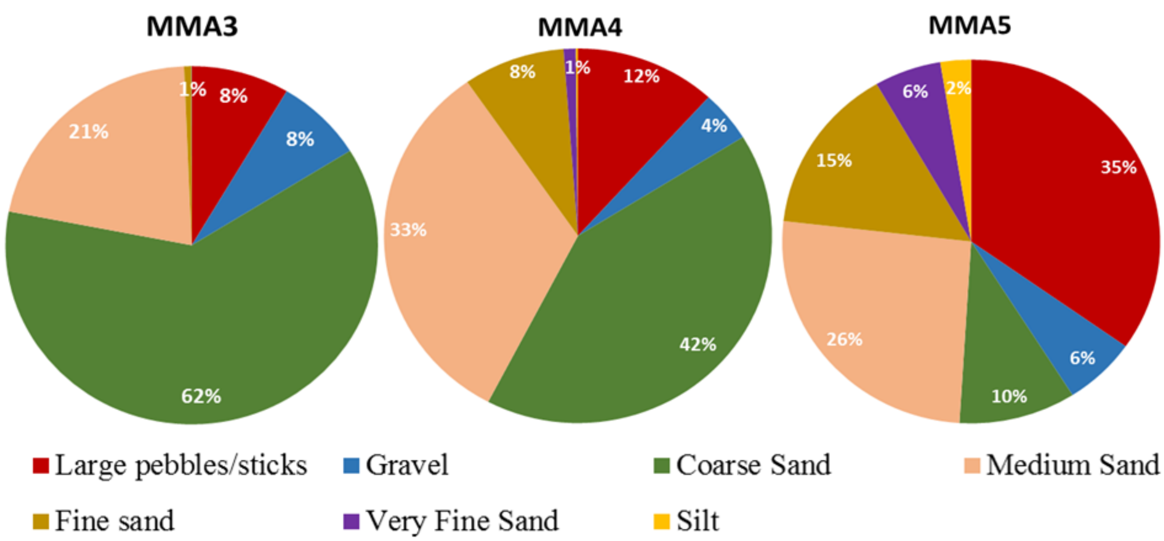

Figure 7: Percent by weight of sediment grain size descriptors for stations MMA3, MMA4 and MMA5.

3.5 Total Organic Carbon Analysis

The other portion of the grab collected for sediment grain size analysis was used for total organic carbon (TOC) analysis. TOC analysis could not be completed for MMA6 and MMA7 because an insufficient amount of sediment was retrieved due to the abundance of *Mytilus edulis* and cobble. The percent by weight of TOC at the collected stations can be found in Table 6.

Table 6: Percent by Weight of Total Organic Carbon at stations MMA3, MMA4 and MMA5.

Station	TOC (%)
MMA3	0.144
MMA4	0.175
MMA5	0.411

3.6 Mussel Length Measurements

Because MMA6 and MMA7 were located in mussel beds, the length of a subset of the mussels collected in each sample was measured to establish baseline conditions and to monitor mussel length over time. The Northeast Fisheries Science Center recommends subsampling at least 20% of the catch (NEFSC, 2016). Therefore, 20% of the total mussels in each grab were measured, but no fewer than 30 individuals. The average length for the two stations was then calculated (Table 7). A complete list of measurements for the subset can be found in Appendix IV.

Table 7: Average number of mussels per grab and average mussel length for a subset of collected mussels, with accompanying standard deviation.

Station	Average Total Number	Average Number Measured	Average length (cm)
MMA6	121±67	33	1.63±0.15
MMA7	283±189	61	1.91±0.32

4. Discussion

4.1 Water Quality

After measuring water quality parameters at each station, it was determined that there is no significant difference in temperature, salinity or dissolved oxygen concentrations between surface and deep depths, indicating a well-mixed water column (Table 2). This data and data collected for the Buzzards Bay Citizens' Water Quality Monitoring Program demonstrates dissolved oxygen concentrations greater than 6.0mg/L, which is consistent with coastal and marine waters classified for use as excellent habitat for fish and other aquatic life and for primary and secondary contact recreation (Massachusetts Department of Environmental Protection, 2013). While all stations exhibited high dissolved oxygen concentrations, MMA5, located in a *Crepidula fornicata* bed, had the highest dissolved oxygen concentrations (Figure 3). MMA5 was sampled two weeks after the other stations and the water temperature had dropped by about 6°C. Colder water holds more oxygen, so the percent saturation of dissolved oxygen at MMA5 was similar to MMA6 and MMA7 and slightly lower than MMA3 and MMA4.

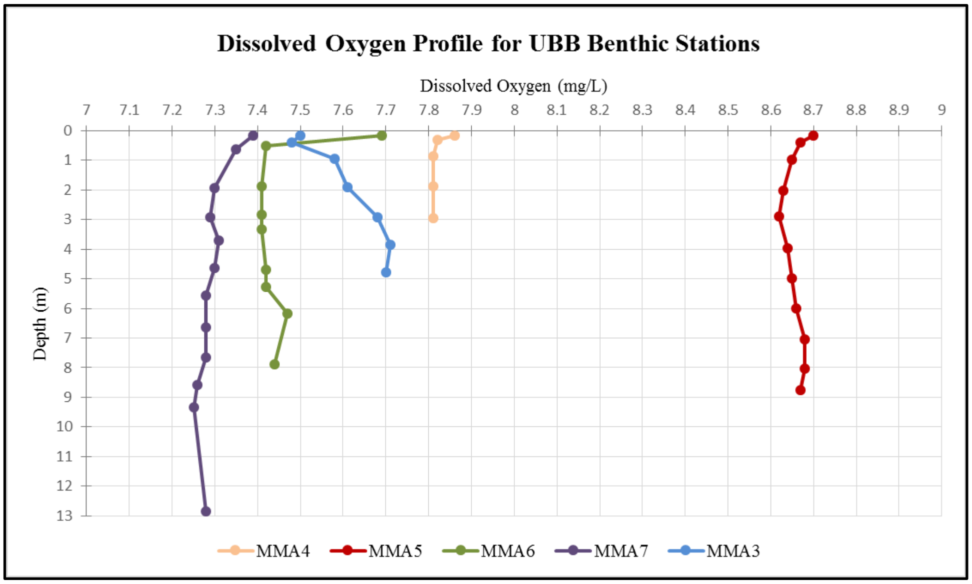

Figure 8: Depth profile for dissolved oxygen concentrations at benthic monitoring stations

4.2 Benthic Community Structure

Four different benthic environments were observed in Upper Buzzards Bay: a *Mytilus edulis* bed, a *Zostera marina* L. community, a sand flat with coarse sand bottom, and a *Crepidula fornicata* community. Stations located in the *Zostera marina* L. and *Crepidula fornicata* communities (MMA4 and MMA5) demonstrate significantly higher biological diversity compared to stations located in the *Mytilus edulis* beds (MMA6 and MMA7). This was expected as *Zostera marina* L. communities are productive plant communities that serve as critical habitats for many ecologically and economically important fish and shellfish species (Neckles, 2015). In addition, *Crepidula fornicata*, while an invasive species, enhances local species diversity because their calcareous shells increase heterogeneity and topographic complexity of soft sediment bottoms and their filter feeding activity increases organic enrichment of the sediment through the excretion of large amounts of particulate biodeposits (Androuin et al, 2018).

When comparing the stations' total organisms, MMA5 had statistically greater numbers than MMA3, MMA4, and MMA6. However, there was not a statistically significant difference in total organisms between MMA5 and MMA7. When examining total number of species though, MMA5 and MMA7 are statistically different. The lack of significant difference between the two stations' total organisms can be attributed to the large quantity of *Mytilus edulis* present at MMA7 (Figure 9).

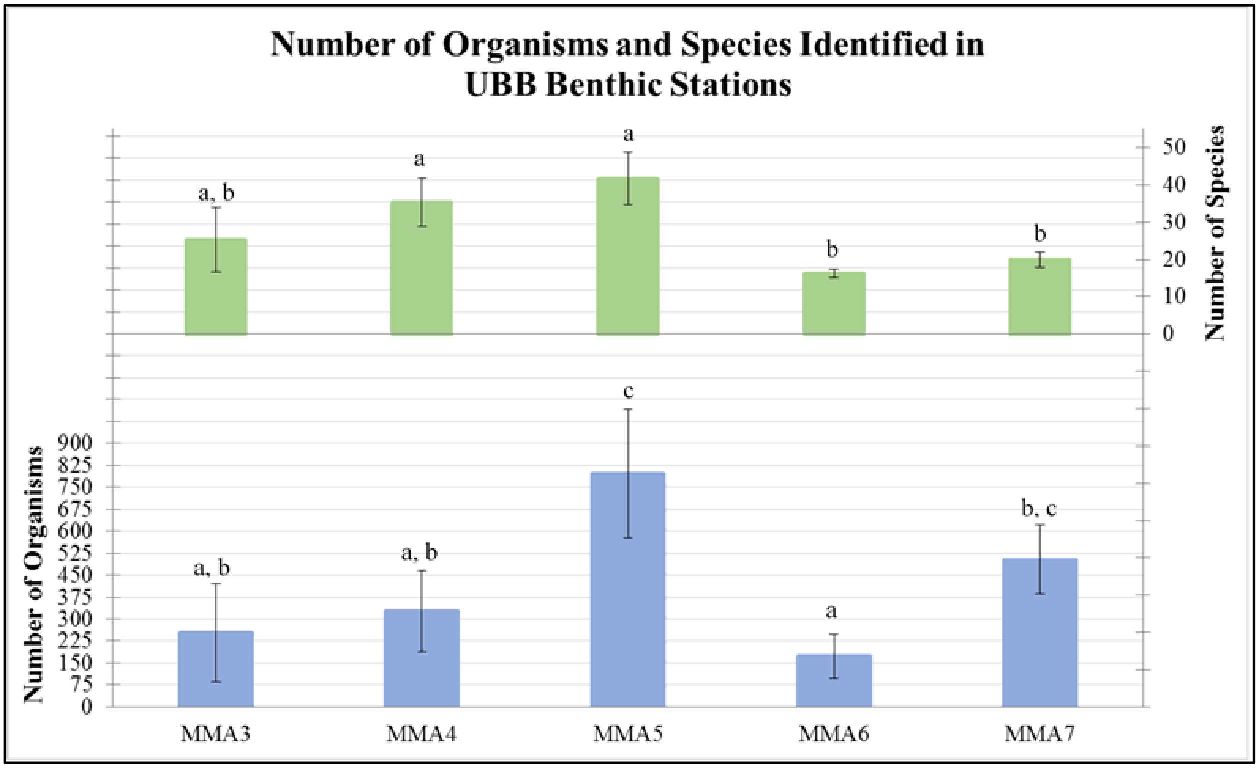

Figure 9: Average number of organisms and species for each benthic monitoring station. Letters indicate significant difference as determined with a two-sample t-test, α=0.05

Diversity indices were calculated for each sample, and then averaged to represent each station to use as a quantifiable measure of the biological quality of the stations' community structure (Simboura and Zenetos, 2002). Shannon-Wiener and Margalef's species richness indices are the most commonly used diversity indices in the assessment of pollution in marine benthic communities (Simboura and Zenetos, 2002). However, because they are both dependent on sample size and habitat type (Simboura and Zenetos, 2002), a calculated number for one area can mean something different for another area. Because there is not a standard for diversity indices, they will be used as a baseline for future comparison.

Based on the diversity indices calculated in Table 3 and Table 4, MMA4 and MMA5 generally had the highest numbers while MMA6 and MMA7 had the lowest.

In addition to diversity indices, it is important to note the presence of indicator species. *Mediomastus ambiseta* is an indicator of an organic rich environment (Howes *et al.*, 2014), and was found to be dominant at MMA5 and second most abundant at MMA4. While the presence of this organism indicates an organic rich environment, it does not signify a contaminated environment (Howes *et al.*, 2014). The stress indicator species, *Cyathura polita* (Howes *et al.*, 2014), was found only at station MMA3 and only one organism was present.

4.3 TOC and Sediment Grain Size

Hyland *et al.* (2000) found that TOC levels below 0.05%-0.1% and above 3.0% were related to decreased benthic abundance and biomass. At very low TOC levels, little food is available for consumers resulting in a low biomass community, and at very high TOC levels, enhanced sediment respiration rates lead to oxygen depletion and accumulation of potentially toxic reduced chemicals (Van Dolah *et al.*, 2006). The three sampling stations where TOC analysis was completed were in the range of healthy TOC content. MMA5 has the highest percent TOC by weight (0.411%), which was more than twice the amount at both MMA3 and MMA4 (Table 6).

Sediment composition influences benthic community structure, the exchange rates of gases and nutrients between the water column and seafloor, and the bioavailability of nutrients and contaminants to resident fauna (Gray, 1974; Graf, 1992). Sediment that has more silt and clay tends to reduce the movement of gases and nutrients and retain more contaminants than sandier sediments (Van Dolah *et al.*, 2006). The three stations where sediment grain size analysis was performed have a silt composition of less than or equal to 2% (Figure 7). MMA3 is primarily composed of coarse sand, MMA4 a mixture of coarse sand and medium sand, and MMA5 large pebbles/sticks and medium sand. Through video and picture observation, MMA6 and MMA7 was primarily composed of shells/shell fragments and cobble. Considering the low amounts of silt/clay in the sediment, it is assumed that there is a healthy exchange of gases and nutrients between the sediment and water column and contaminants aren't being retained at these stations.

4.4 Comparison with Previous Studies

To understand how these results relate to other areas in Buzzards Bay with similar benthic substrate, benthic survey research completed by William Hubbard under the Coastal America Foundation was reviewed (Hubbard, 2016). Hubbard's research re-occupied Howard Sanders benthic stations from 1955 (Sanders, 1958) to examine changes in the benthic community structure (Hubbard, 2016). Station locations can be found in Figure 10, which also depicts the spatial relationship to this research's stations. The stations sampled in this study are much closer to shore than the locations of previous studies.

Though an insufficient amount of sediment was retrieved at MMA6 and MMA7 to perform the sediment grain size analysis due to the abundance of *Mytilus edulis* and cobble, an estimate was used to allow the inclusion of these sites in the comparison with previous studies. The dominant species at MMA6 and MMA7, *Mytilus edulis*, constituted 70% (MMA6) and 56% (MMA7) of the population with the average shell length of between 1.63cm and 1.91cm (Table 7). After reviewing still photos of the grabs prior to processing (Figure 11), we estimate that over 75% of the grab falls under the Large Pebbles/Sticks category.

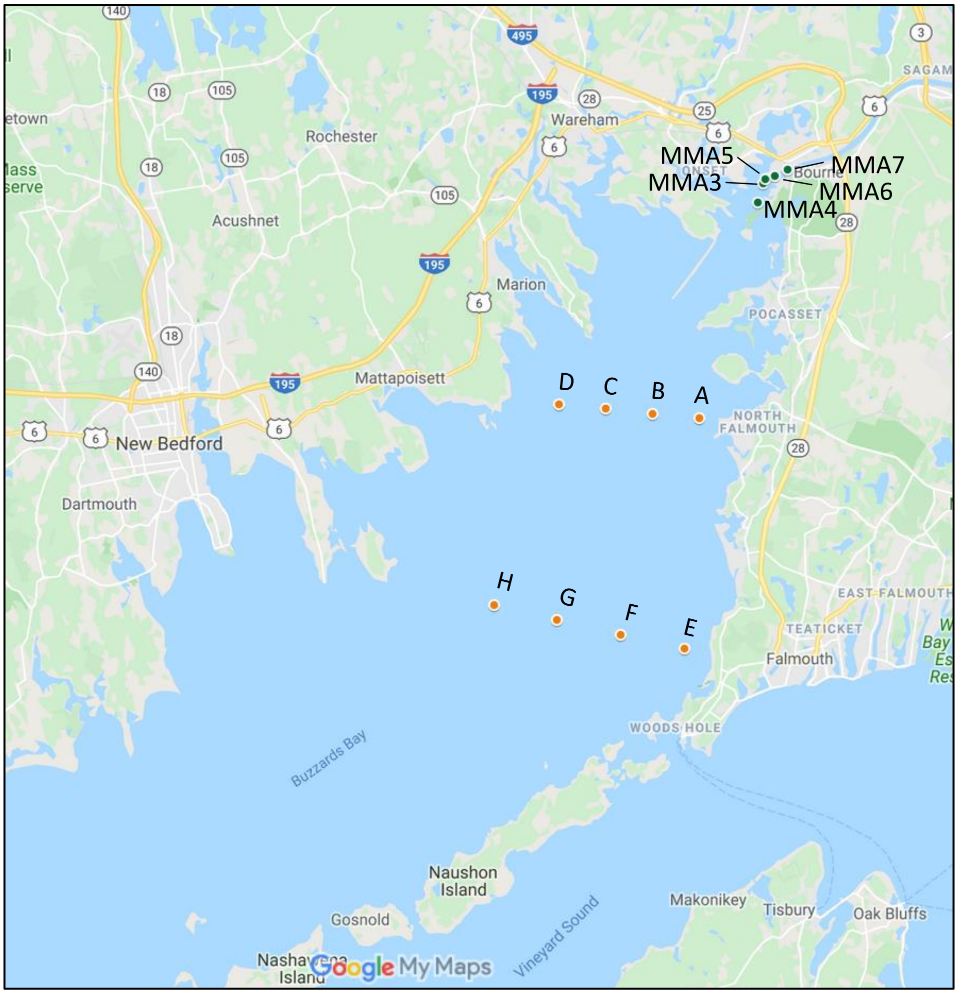

Figure 10: Locations of stations A-H (orange dots) that were sampled by Sanders in 1955 and the Coastal America Foundation in 2011/2012 as well as stations MMA3-7 sampled in 2018 as a part of this study (green dots).

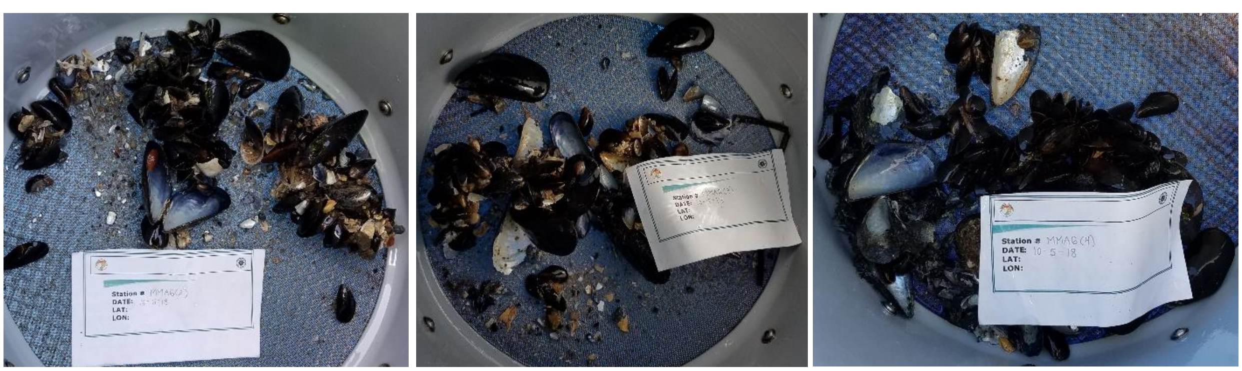

Figure 11: Still photos of three replicate benthic grabs taken at MMA6.

There were two sets of sites that had similar sediment composition profiles. Stations MMA3 and MMA4 in this study had sediment grain size characteristics similar to Hubbard's stations CAF-C and CAF-E (Table 8). Stations MMA6 and MMA7, located at mussel beds in the Cape Cod Canal, were estimated to be dominated by the Large Pebbles/Sticks category, which is similar to Hubbard's stations CAF-A and CAF-D (Table 8).

Table 8: Sediment grain size analysis results from Hubbard's 2016 study and current study. The sieves used differed slightly between studies. The size classes are defined as: Large Pebbles/Sticks (>4 mm this study, >4.75mm Hubbard); Gravel (2-4mm this study, 2-4.75mm Hubbard); Coarse Sand (0.5-2mm this study, 0.425-2mm Hubbard); Very-Fine/Fine/Medium Sand (0.063-0.5mm this study, 0.075-0.425mm Hubbard); and Silt/Clay (<0.063mm this study, <0.075mm Hubbard). Stations highlighted the same color share a similar sediment composition distribution.

Station	% Large Pebbles/Sticks	% Gravel	% Coarse sand	% Very-Fine/Fine/ Medium Sand	% Silt/Clay
Hubbard 2016					
CAF-A	63.74	2.45	11.08	7.80	14.92
CAF-B	0.32	0.18	3.51	29.38	66.61
CAF-C	2.87	3.05	60.09	29.55	4.45
CAF-D	74.12	1.34	2.69	2.86	18.99
CAF-E	9.36	3.36	52.33	33.64	1.32
CAF-F	0.00	0.02	0.95	3.74	95.29
CAF-G	0.01	0.15	23.70	44.70	31.44
CAF-H	0.02	0.08	12.23	29.81	57.87
	This Study				
MMA3	8.52	7.65	61.86	21.92	0.04
MMA4	11.75	4.42	41.49	41.14	1.2
MMA5	34.77	6.22	10.00	40.66	8.35
MMA6	>75	--	--	--	--
MMA7	>75	--	--	--	--

For the sets of stations that had similar sediment grain size characteristics, the number of species, number of individuals, and diversity indices were compared (Table 9). There was good agreement between the stations with similar sediment composition profiles – with student's ttests generally indicating that there were not significant differences between the values from this study in comparison to those found by Hubbard (2016). The exceptions to that were that the Shannon-Weiner Index and Simpson Index for MMA4 were significantly different from those indices for CAF-C and that MMA7 had a significantly different number of individuals than either CAF-A or CAF-D. Thus, the sediment data collected from this survey of Upper Buzzards Bay compares well with Hubbard's earlier survey of Mid to Upper Buzzards Bay.

Table 9: Comparison data from Hubbard's 2016 study and current study. Stations highlighted the same color share a similar sediment composition distribution.

Station	Total Species	Total Individuals	Margalef's Species Richness	Pielou's Evenness Index	Fisher's Alpha Index	Shannon-Weiner Index	Simpson Index
CAF-C	25±3	324±138	4.16±0.506	0.636±0.073	6.46±1.142	2.04±0.256	0.747±0.042
CAF-E	22±8	658±396	3.39±1.07	0.525±0.192	4.81±1.73	1.60±0.55	0.661±0.142
MMA3	25±9	253±167	4.76±1.92	0.715±0.216	10.05±7.27	2.32±0.88	0.805±0.204
MMA4	35±7	327±139	5.99±1.09	0.768±0.040	10.43±2.69	2.73±0.10	0.900±0.012
CAF-A	16±1	138±18	3.12±0.309	0.649±0.113	4.86±0.691	1.81±0.306	0.696±0.128
CAF-D	13±3	65±30	2.94±0.66	0.772±0.099	5.39±1.98	1.97±0.34	0.789±0.100
MMA6	16±1	174±75	3.03±0.45	0.499±0.121	4.65±1.07	1.40±0.37	0.531±0.138
MMA7	20±2	504±118	3.07±0.39	0.524±0.152	4.21±0.70	1.58±0.50	0.623±0.193

5. Conclusion

This study of Upper Buzzards Bay benthic environments showed that there are a variety of different communities and substrates within the study area. These included areas consisting of a *Mytilus edulis* bed, a *Zostera marina* L. community, a sand flat with coarse sand bottom, and a *Crepidula fornicata* community. While each area is unique, the benthic survey demonstrated that all areas supported benthic macrofauna communities and that low dissolved oxygen concentrations were not encountered. The diversity indices provide insight on the relative habitat suitability of the different benthic environments. Stations located outside of the canal (MMA3, MMA4, MMA5) generally had higher diversity indices compared to stations located within the canal (MMA6 and MMA7). This is consistent with the extremely strong currents through the canal that scour sediments making it a challenging environment for benthic species.

6. References

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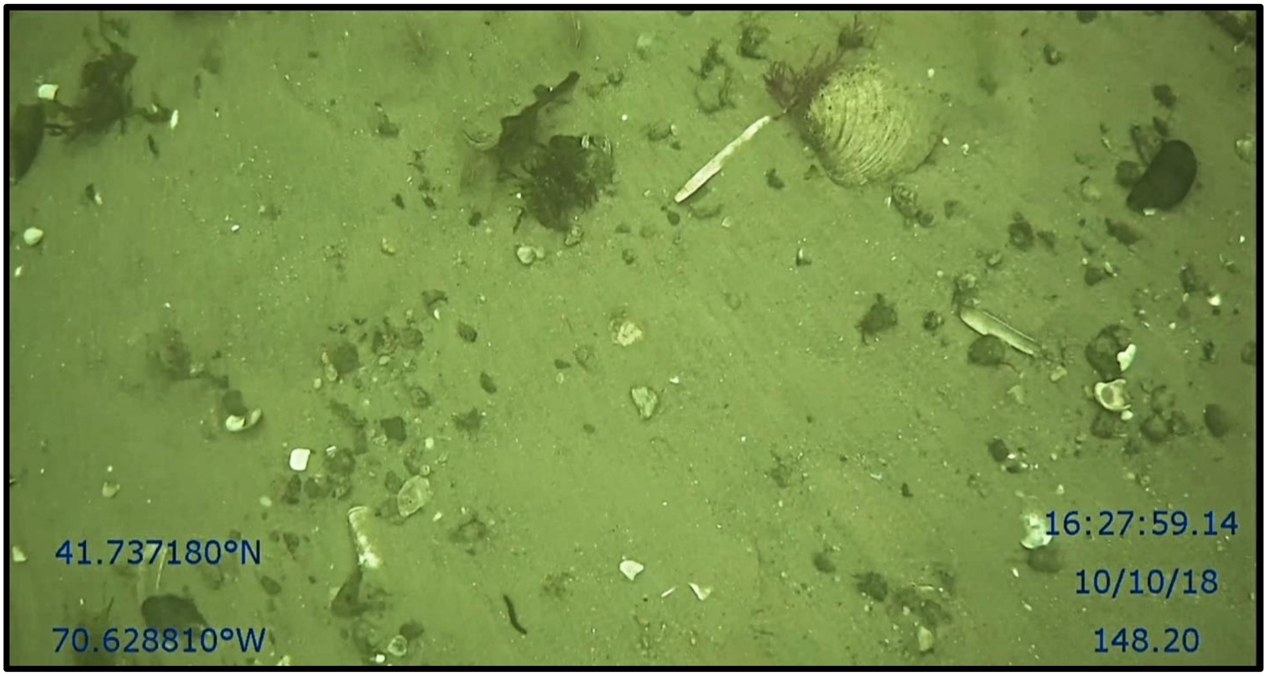
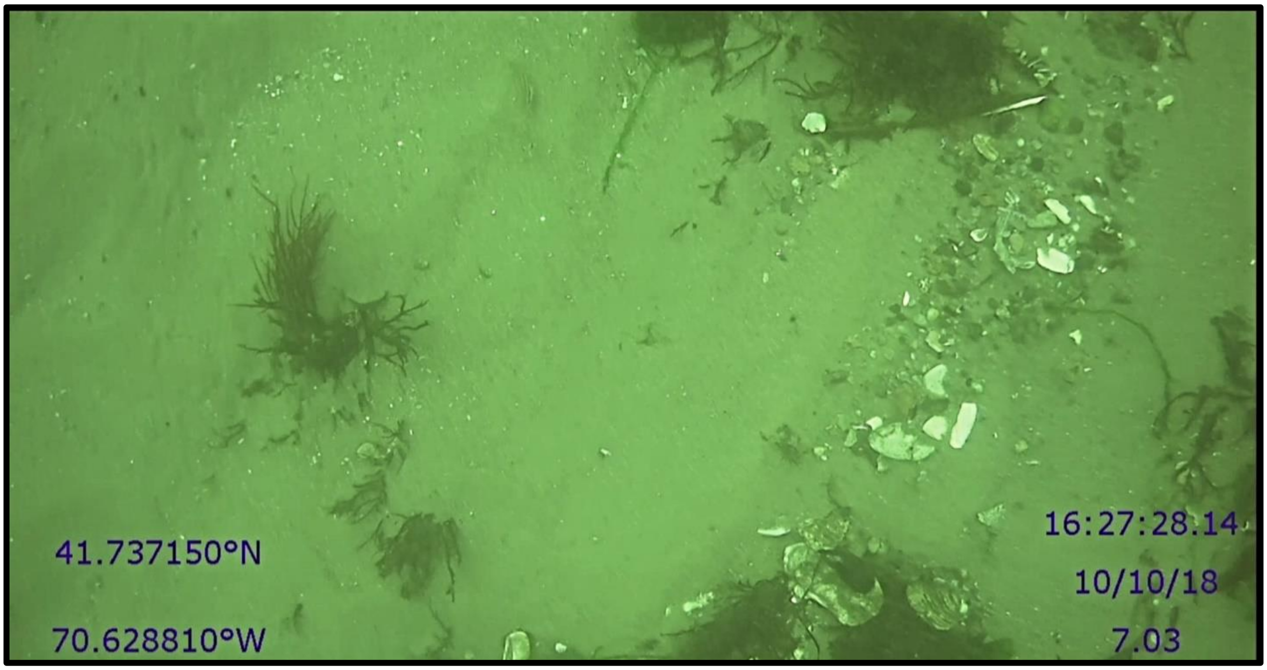
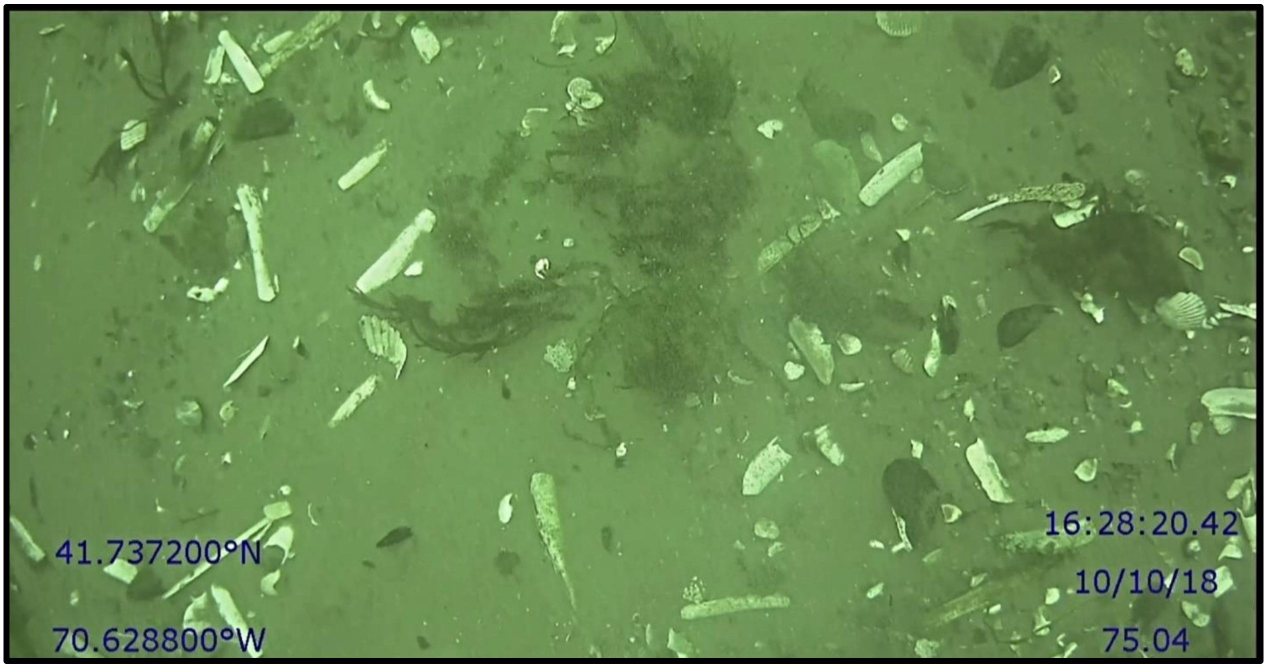
Appendix I. Additional photos of benthic stations

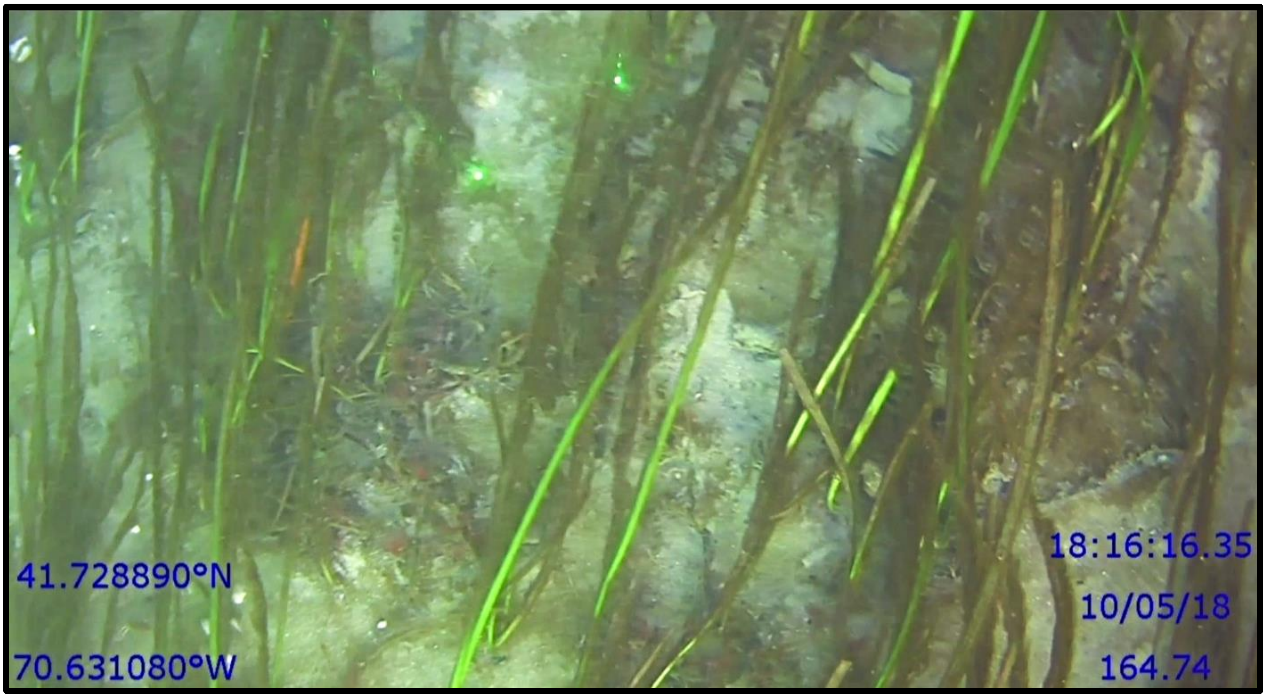
Station MMA3

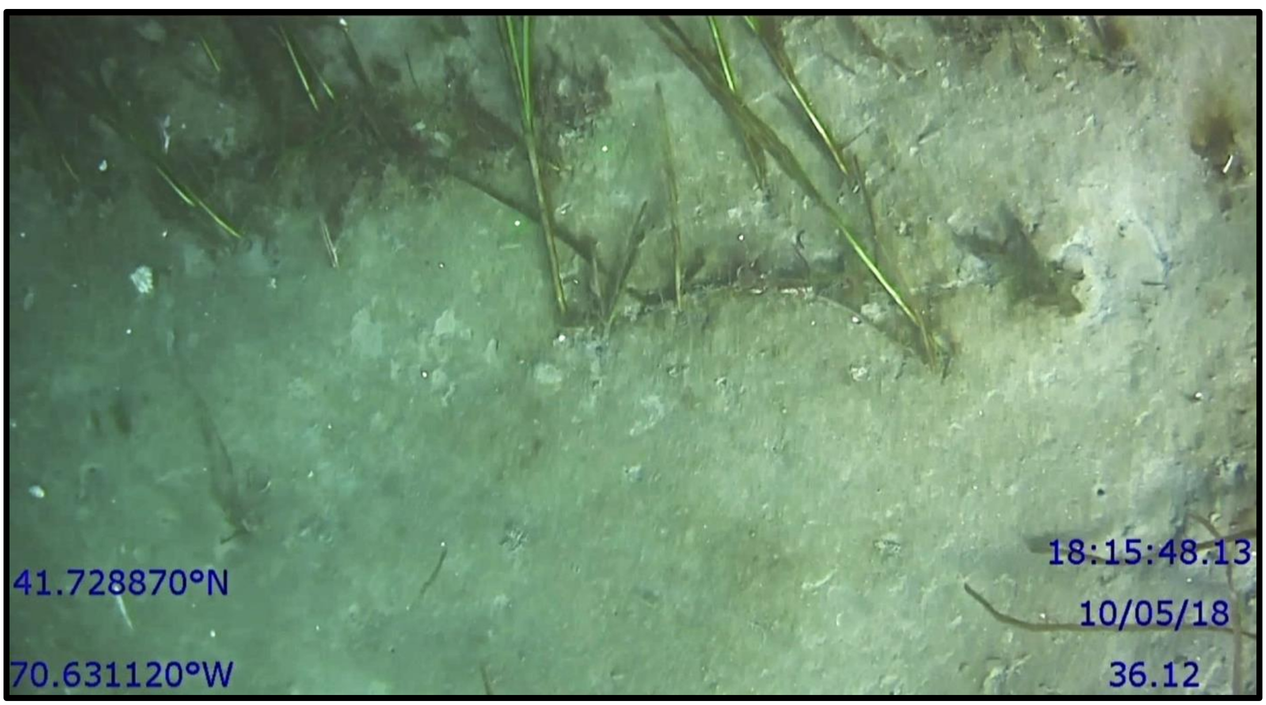




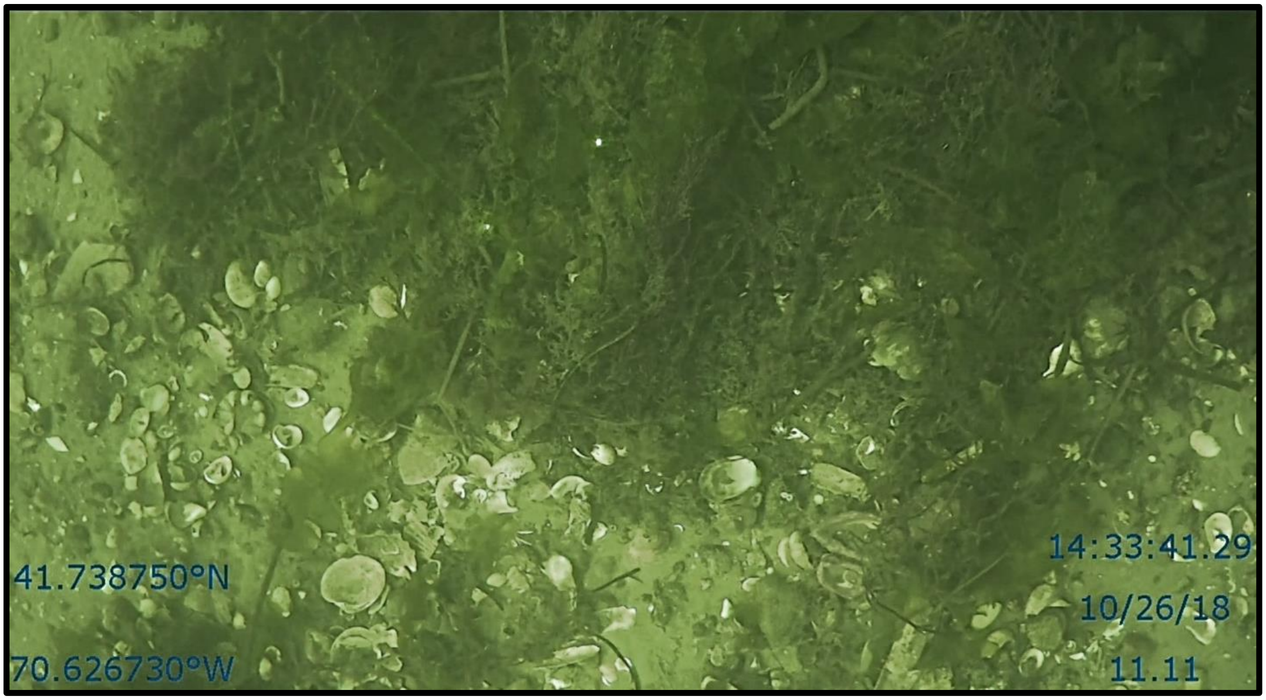


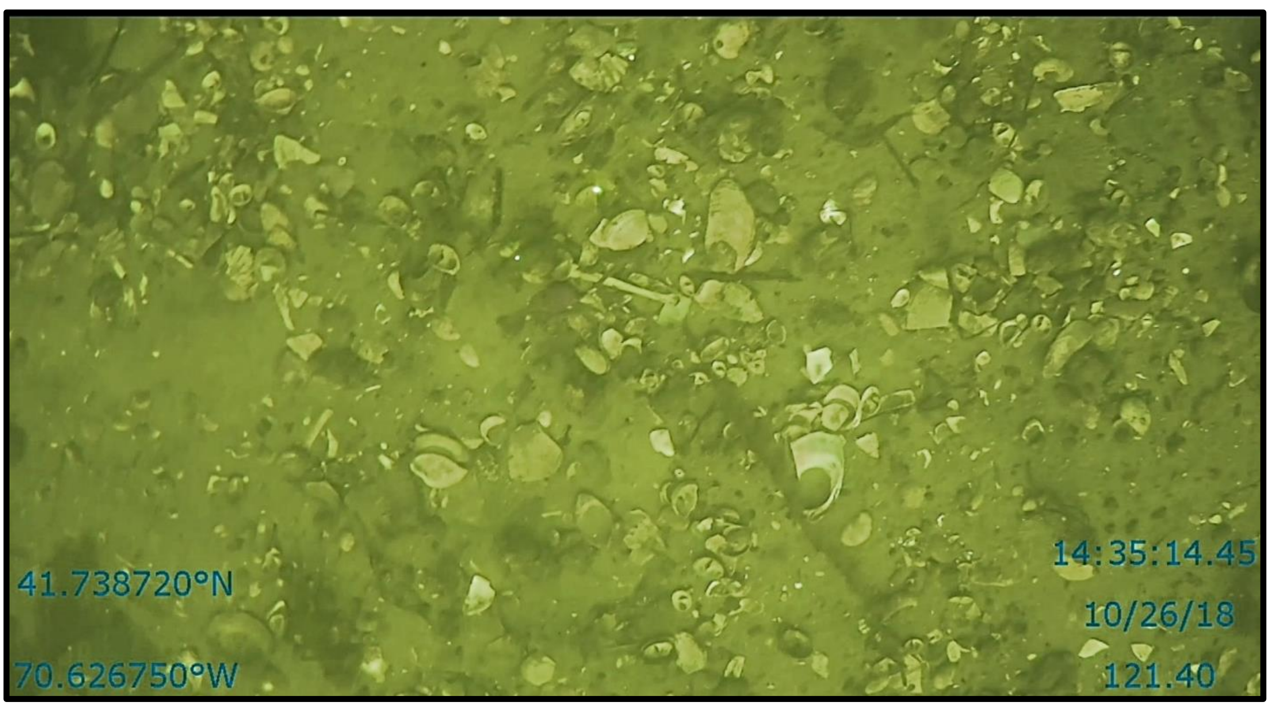
Station MMA4

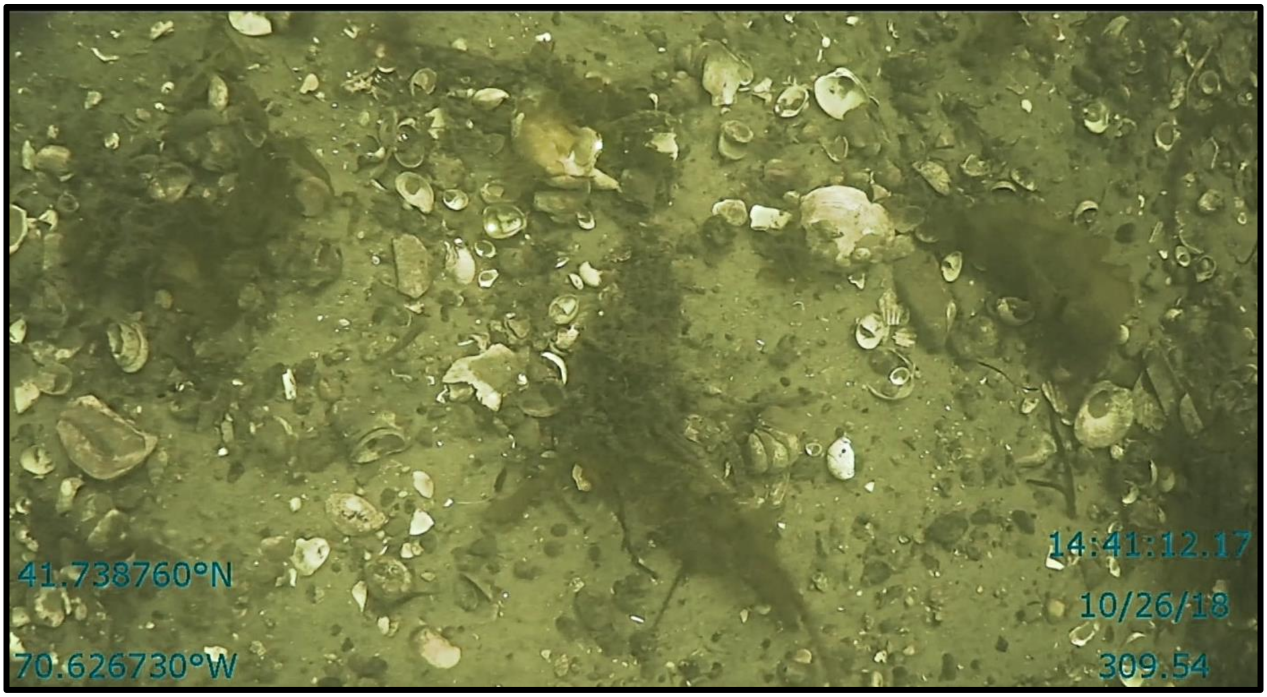

41.728890°N
70.631080°W
18:16:16.35
10/05/18
164.74


41.728870°N
70.631120°W
18:15:48.13
10/05/18
36.12

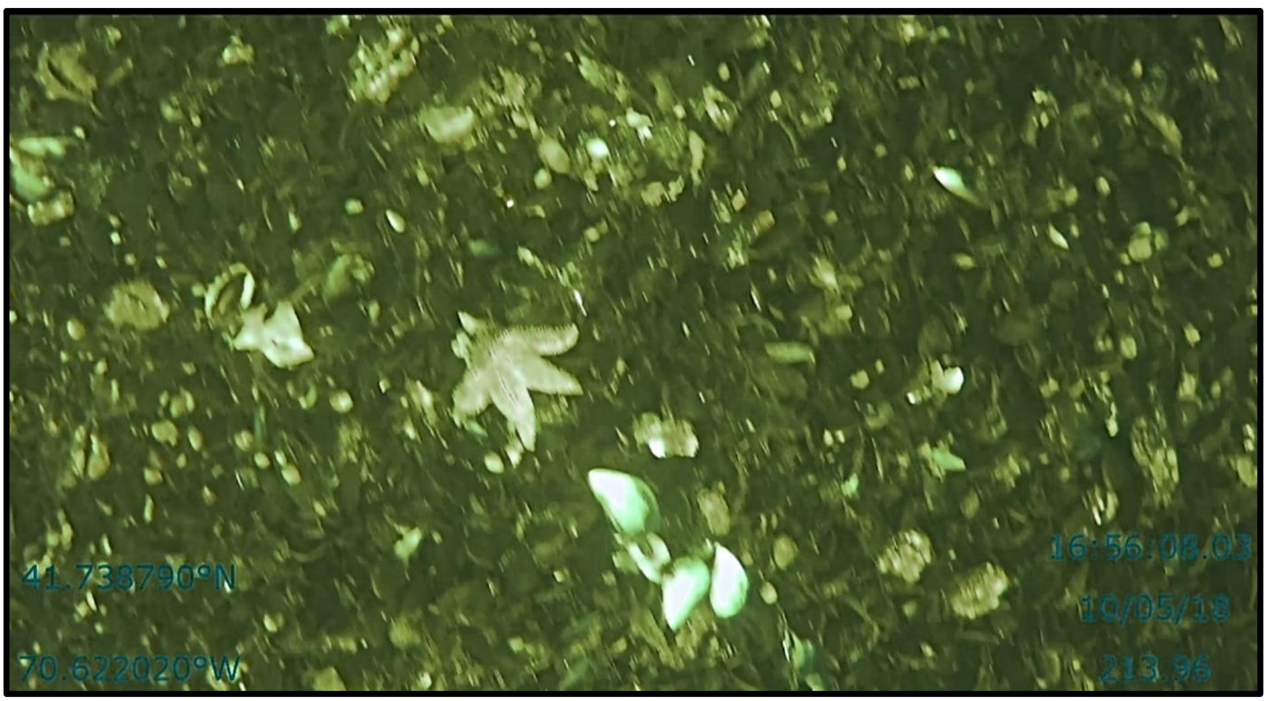
Station MMA5

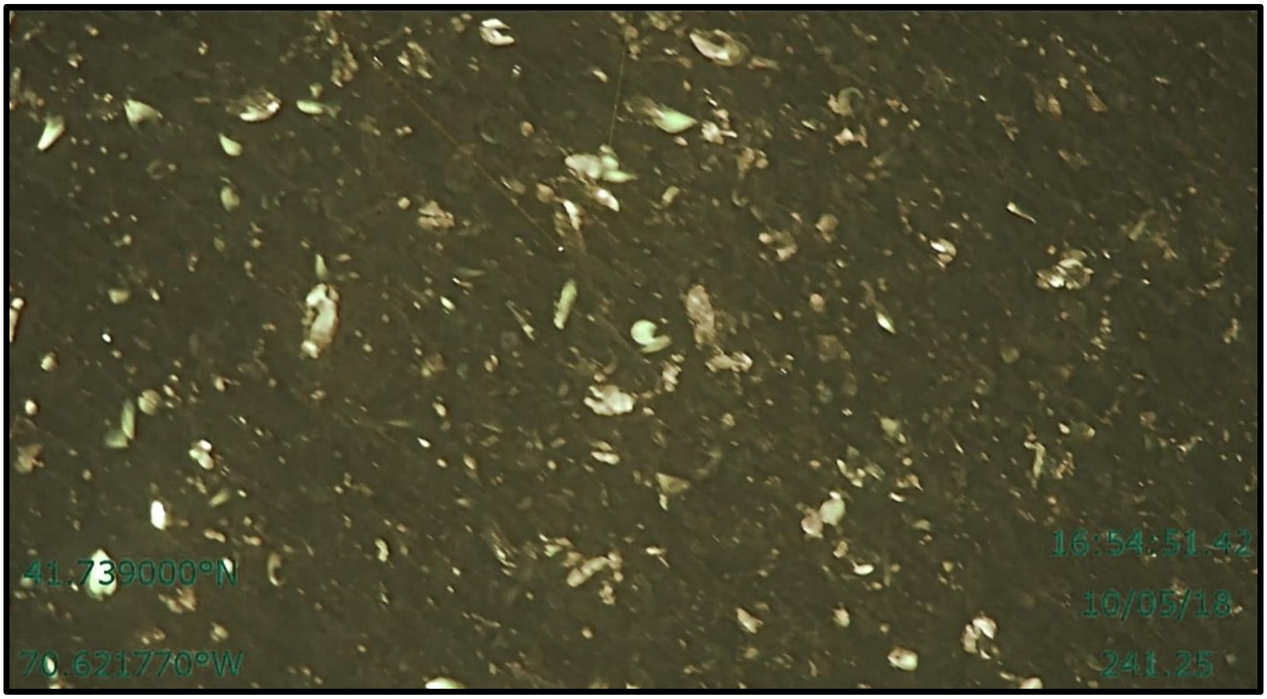






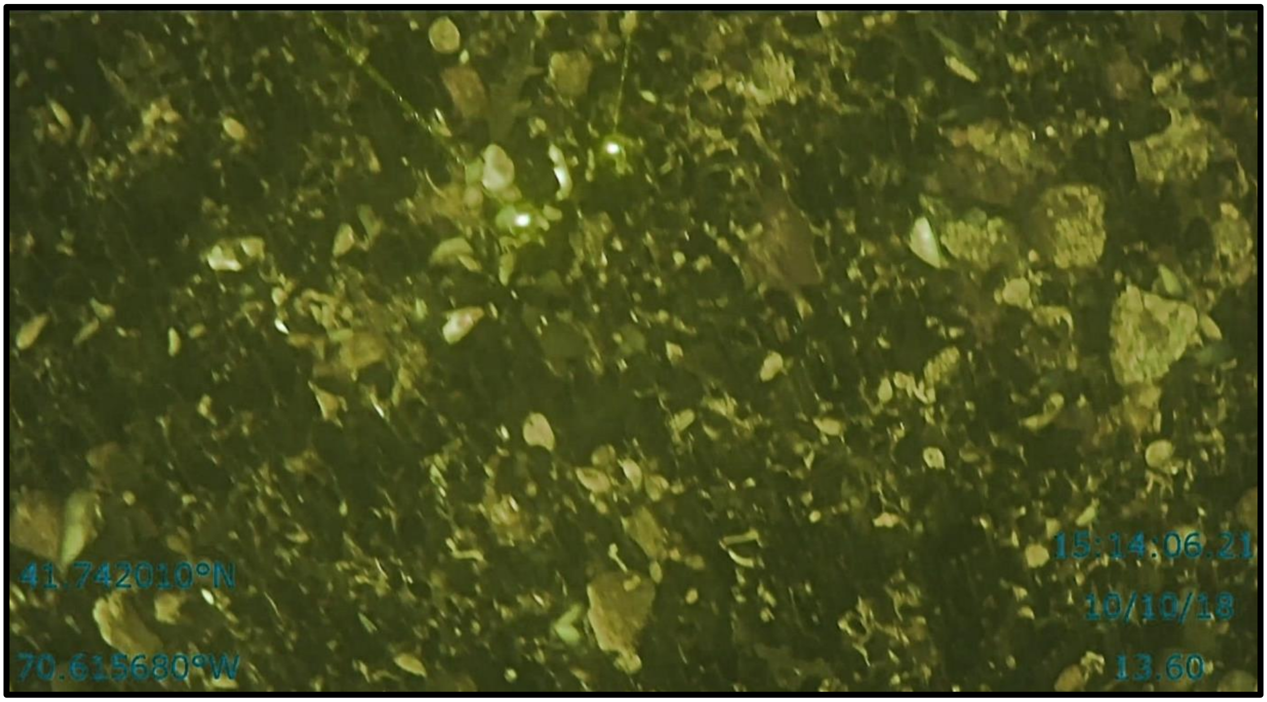
Station MMA6

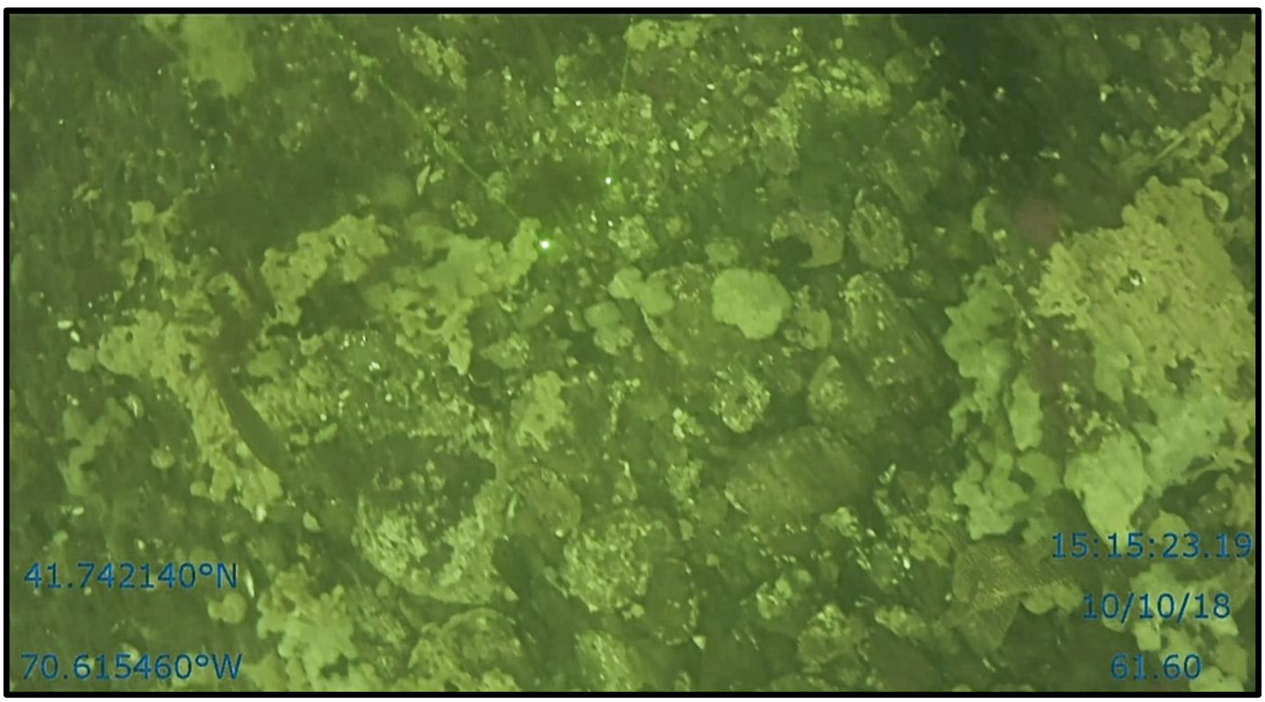


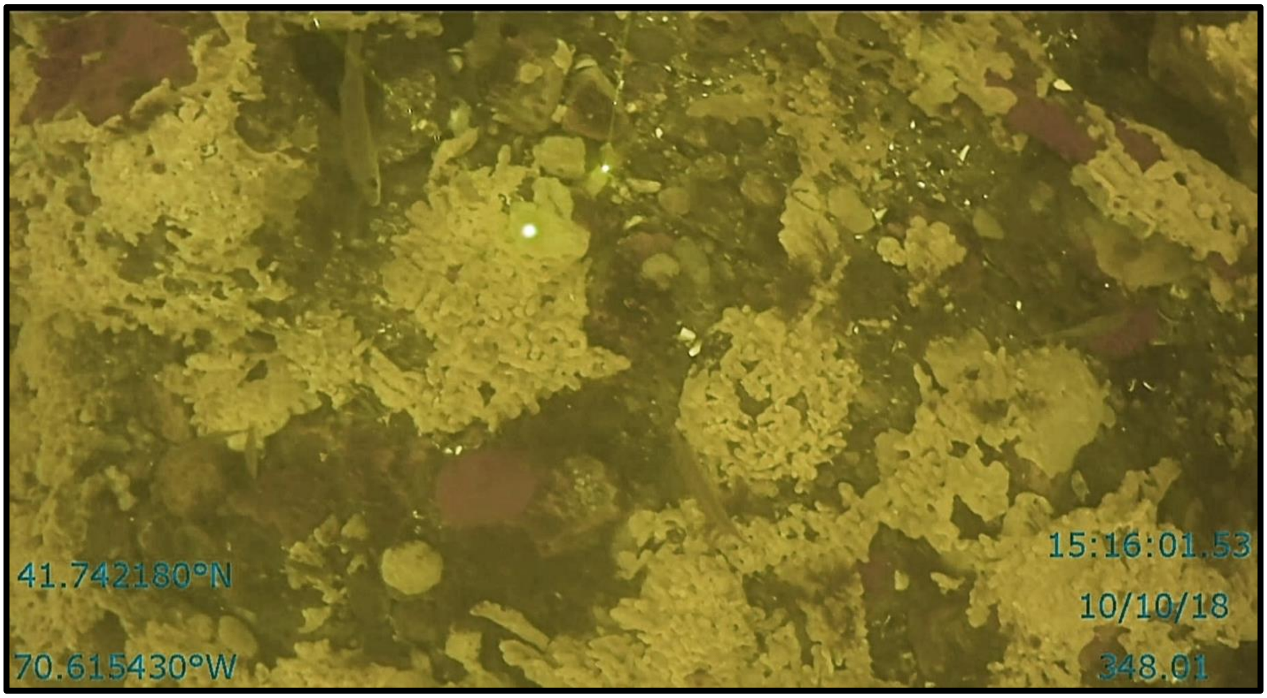

41.739000°N
70.621770°W
16:54:51.42
10/05/18
241.25


41.738540°N
70.622280°W
16:57:21.18
10/05/18
220.07

Station MMA7






41.742180°N
70.615430°W
15:16:01.53
10/10/18
348.01

Appendix II. Water Quality Measurements

Station	Date	Total Depth (m)	Sample Depth (m)	Time	Temper-ature (°C)	Dissolved Oxygen (mg/L)	Dissolved Oxygen (% sat)	Salinity	pH
MMA3	10/26/2018	9.02	0.15	12:17	18.7	7.50	96.4	31.24	7.84
			0.39	12:17	18.6	7.48	95.9	31.21	7.81
			0.95	12:18	18.7	7.58	97.3	31.21	7.81
			1.90	12:18	28.7	7.61	97.7	31.21	7.82
			2.91	12:19	18.7	7.68	98.7	31.21	7.82
			3.86	12:19	18.8	7.71	99.2	31.21	7.83
			4.79	12:20	18.7	7.70	99.0	31.21	7.83
MMA4	10/5/2018	3.05	0.15	14:07	18.0	7.86	99.6	31.58	8.35
			0.30	14:08	18.0	7.82	99.0	31.58	8.34
			0.85	14:09	18.1	7.81	99.0	31.57	8.34
			1.86	14:10	18.1	7.81	99.0	31.57	8.33
			2.94	14:10	18.1	7.81	99.0	31.57	8.33
MMA5	10/26/2018	9.02	0.15	10:26	11.6	8.70	95.6	29.4	7.79
			0.40	10:27	11.6	8.67	95.3	29.4	7.78
			0.96	10:29	11.6	8.65	95.1	29.4	7.78
			2.02	10:30	11.6	8.63	94.9	29.41	7.78
			2.88	10:31	11.6	8.62	94.8	29.41	7.77
			3.96	10:32	11.6	8.64	95.1	29.41	7.78
			4.98	10:32	11.6	8.65	95.2	29.41	7.78
			6.00	10:33	11.6	8.66	95.3	29.41	7.78
			7.04	10:33	11.6	8.68	95.4	29.41	7.78
			8.04	10:34	11.6	8.68	95.5	29.4	7.78
			8.76	10:35	11.6	8.67	95.3	29.4	7.78

Station	Date	Total Depth (m)	Sample Depth (m)	Time	Temper-ature (°C)	Dissolved Oxygen (mg/L)	Dissolved Oxygen (% sat)	Salinity	pH
MMA6	10/5/2018	8.5	0.15	12:33	17.7	7.69	96.7	31.70	8.04
			0.50	12:34	17.7	7.42	93.4	31.70	8.11
			1.88	12:34	17.7	7.41	93.2	31.71	8.13
			2.82	12:35	17.7	7.41	93.2	31.71	8.14
			3.34	12:35	17.7	7.41	93.2	31.71	8.16
			4.69	12:36	17.7	7.42	93.4	31.71	8.18
			5.28	12:37	17.6	7.42	93.3	31.71	8.27
			6.16	12:37	17.7	7.47	94.0	31.71	8.31
			7.89	12:38	17.7	7.44	93.6	31.69	8.20
MMA7	10/10/2018	13	0.15	11:06	18.5	7.39	94.5	31.23	7.80
			0.63	10:58	18.5	7.35	94.0	31.21	7.70
			1.92	10:59	18.5	7.30	93.4	31.21	7.74
			2.92	10:59	18.5	7.29	93.2	31.21	7.75
			3.71	11:00	18.5	7.31	93.4	31.21	7.76
			4.64	11:00	18.5	7.30	93.4	31.21	7.77
			5.57	11:01	18.5	7.28	93.1	31.21	7.77
			6.63	11:01	18.5	7.28	93.0	31.21	7.78
			7.66	11:02	18.5	7.28	93.0	31.20	7.78
			8.57	11:02	18.5	7.26	92.9	31.20	7.78
			9.34	11:03	18.5	7.25	92.7	31.20	7.78
			12.85	11:04	18.5	7.28	93.1	31.21	7.79

Appendix III: Complete List of Identified Species for UBB Benthic Stations

The table below lists the number of individuals of all the species found in each replicate (as indicated by the number in parentheses below the station identification) of each station.

Species	MMA3			MMA4			MMA5			MMA6			MMA7		
	(2)	(3)	(4)	(2)	(3)	(4)	(2)	(3)	(4)	(2)	(3)	(4)	(1)	(2)	(4)
Alitta succinea				2			5	10	5	4	1				
Almyracuma proximoculi				1	2										
Amastigos caperatus				1											
Ampelisca macrocephala						1									
Ampithoe longimana	5														
Ampithoe rubricata	5							5					1		
Ampithoe valida								2							
Anadara transversa							1								
Anomia simplex									1						
Aricidea cerrutii						1									
Astyris lunata		7	5				149	55	57	3	6	9	33	38	46
Callipallene brevirostris	3														
Cancer borealis						1									
Caprella unica								1							
Chaetozone setosa					38	15									
Chiridotea almyra			1	1	2	1									
Cirratulus grandis					1										
Clymenella zonalis						1									
Costoanachis avara													1		
Crassicorophium bonellii							3	6	1	2		2	13		
Crassostrea virginica				2											
Crepidula fornicata	1	2			2	4	123	22	100	3	11	3	12	23	15
Cyathura polita		1													
Deflexilodes intermedius					1										
Dodecaceria coralii				28	17	50									
Drilonereis longa			1												
Drilonereis magna		1		9	7	9									
Dyspanopeus sayi								5	12		3	3	7		9
Edotia triloba				2											
Elasmopus levis								1							1
Ergaea walshi							4		2						
Erichsonella filiformis	1							7	1						
Ericthonius brasiliensis	8		1								4				
Erinaceusyllis erinaceus	32	11	4		5	2	29	26	66	3			10		2
Eumida sanguinea			1	1		2	7	6	9		1	2		1	
Eusyllis fragilis														1	
Exogone dispar			2		4		7		8						

Species	**MMA3**			**MMA4**			**MMA5**			**MMA6**			**MMA7**		
	(2)	**(3)**	**(4)**	**(2)**	**(3)**	**(4)**	**(2)**	**(3)**	**(4)**	**(2)**	**(3)**	**(4)**	**(1)**	**(2)**	**(4)**
Exogone verugera				11	29	36		2							
Globosolembos smithi	2						1	1							
Glycera americana								1							
Glycera capitata					1	1									
Glycera dibranchiata			2	17	12	6									
Gyptis vittata								1	2					1	
Harmothoe extenuata									1		1	1	6	3	8
Hediste diversicolor						1									
Hemigrapsus sanguineus														1	1
Hexapanopeus angustifrons							3								
Hydriodes dianthus							1								
Hypereteone heteropoda	4			3	4	4		5	1						
Idotea balthica	16	2	2			2	14	26	17	10	7	25	111	49	47
Idotea metallica							2	6	4	4	1	4	10		5
Idunella clymenellae															1
Ischyrocerus anguipes							1	29	4	1		4	23		
Laonice cirrata	1			15	17	11									
Leitoscoloplos acutus						1									
Leitoscoloplos fragilis	44	44	8	2	3	1		1			1				
Lembos websteri							33	219	66						
Lepidonotus squamatus								1		1		4	6	5	3
Limecola balthica			1						1						
Limnoria lignorum					1										
Littorina obtusata							1								
Lysianopsis alba	1						11	27	26						
Marenzelleria viridis				1											
Mediomastus ambiseta	67	1	4	42	106	19	193	100	510						
Melita nitida							2	6	1	1			1		
Mercenaria mercenaria				2		2		1	2						
Microdeutopus anomalus							2	21	10						
Microdeutopus gryllotalpa	3		2	1	2	2		5		1	1		2	5	
Microphthalmus aberrans	29	204	3												
Monocorophium insidiosum	15		1	3			3	3		2	2		72	12	2
Mya arenaria	3			3	22	5		1	1						
Myrianida prolifera															1
Mysis stenolepis							9	12	4						
Mystides borealis														1	
Mytilus edulis								2		109	61	194	87	299	464
Neanthes acuminata	16	7	3				8	8	8	1	1				
Neoamphitrite figulus			1											1	
Nephtys picta			1	4		1									
Notomastus latericeus									11						

Species	MMA3			MMA4			MMA5			MMA6			MMA7		
	(2)	(3)	(4)	(2)	(3)	(4)	(2)	(3)	(4)	(2)	(3)	(4)	(1)	(2)	(4)
Nucella lapillus							2	3	3				1		
Nucula proxima	1				1	1	1		4						
Odontosyllis fulgurans							1	1	2						
Oxyurostylis smithi				12	8	4									
Pagurus longicarpus	2														
Pagurus pollicaris						1									
Paracaprella tenuis	14		1	2	1			2							1
Parapionosyllis longicirrata	71	35	10	10	49	2		6	13						2
Parexogone hebes	4	1			1	1	1		3						
Paucibranchia bellii		1													
Phascolopsis gouldii			1												
Phoxocephalus holbolli			1												
Phyllodoce groenlandica				2		1									
Pista maculata								1							
Platynereis dumerilii								1						1	
Polydora websteri	3	2				1	5	1	7			3	1		
Polyphysia crassa	1			1	2	1									
Prionospio heterobranchia					1			2							
Prionospio steenstrupi	4	3		5	4	6	17	3	24						
Proceraea cornuta	1								2	2			2		1
Ptilanthura tenuis					1										
Pygospio elegans				7		3	1	2	1						
Rhepoxynius epistomus	7	1	2		2	2									
Rhithropanopeus harrisii							16	5	14	3	4	1		27	9
Salvatoria clavata				23	91	59			2		1		1		
Schistomeringos rudolphi	2				25	4									
Seila adamsii									1						
Spiophanes bombyx	1		1			4	2	2							
Stenothoe minuta	6		2									1			
Streblospio benedicti						1									
Syllis gracilis							1	2							
Tharyx setigera					12	12									
Tritia trivittata			1	2	4	6	23	5	42			1	1	8	7
Tumidotheres maculatus	1		1							3	6		1	1	9
Unciola serrata								1							

Appendix IV: Mussel Measurements

Table A4.1 Station MMA6, Replicate 2 Mussel Measurements

Assigned Number	Length (cm)
1	4.80
2	2.20
3	0.80
4	1.10
5	0.60
6	1.55
7	1.60
8	2.05
9	2.00
10	2.15
11	0.40
12	1.75
13	0.55
14	1.70
15	1.95
16	2.20
17	4.55
18	1.60
19	0.60
20	1.30
21	1.50
22	2.50
23	2.45
24	0.75
25	1.60
26	2.50
27	2.40
28	1.00
29	1.80
30	0.70

Table A4.2 Station MMA6, Replicate 3 Mussel Measurements

Assigned Number	Length (cm)
1	1.55
2	2.05
3	0.55
4	1.55
5	1.85
6	2.20
7	0.85
8	1.65
9	2.20
10	5.20
11	1.35
12	1.55
13	1.95
14	0.90
15	1.25
16	2.05
17	1.25
18	1.60
19	1.90
20	2.10
21	1.80
22	1.40
23	0.95
24	1.20
25	2.50
26	1.20
27	0.80
28	0.95
29	1.75
30	1.40

Table A4.3 Station MMA6, Replicate 4 Mussel Lengths

Assigned Number	Length (cm)	Assigned Number	Length (cm)
1	0.55	31	2.25
2	1.40	32	1.55
3	1.90	33	1.25
4	1.40	34	1.80
5	1.35	35	3.60
6	1.15	36	1.75
7	1.75	37	1.40
8	1.85	38	0.80
9	0.45	39	1.80
10	1.00		
11	0.95		
12	1.05		
13	1.20		
14	0.90		
15	1.30		
16	1.10		
17	1.65		
18	1.80		
19	0.95		
20	1.00		
21	1.20		
22	1.70		
23	1.90		
24	1.60		
25	2.40		
26	0.45		
27	1.40		
28	2.00		
29	1.70		
30	1.90		

Table A4.4 Station MMA7, Replicate 1 Mussel Lengths

Assigned Number	Length (cm)
1	1.95
2	2.15
3	2.05
4	2.30
5	2.05
6	2.30
7	1.40
8	1.25
9	1.20
10	2.15
11	2.30
12	2.10
13	1.80
14	1.60
15	1.40
16	1.90
17	5.70
18	2.60
19	4.00
20	1.70
21	1.95
22	6.60
23	1.40
24	1.15
25	2.30
26	1.85
27	1.55
28	3.65
29	1.55
30	1.90

Table A4.5 Station MMA7, Replicate 2 Mussel Measurements

Assigned Number	Length (cm)	Assigned Number	Length (cm)
1	4.65	31	1.30
2	4.25	32	1.60
3	2.20	33	1.70
4	1.90	34	1.85
5	1.50	35	1.4
6	5.05	36	1.35
7	5.10	37	1.15
8	2.35	38	1.70
9	1.55	39	1.60
10	2.20	40	1.30
11	1.60	41	2.35
12	4.70	42	1.95
13	1.80	43	1.70
14	2.2	44	1.50
15	1.75	45	1.70
16	2.05	46	1.50
17	1.30	47	1.80
18	2.00	48	1.15
19	1.65	49	1.00
20	2.50	50	0.95
21	1.95	51	1.10
22	1.95	52	1.10
23	1.25	53	1.00
24	1.60	54	1.05
25	1.75	55	1.30
26	1.30	56	1.10
27	1.45	57	1.25
28	1.65	58	1.45
29	2.00	59	1.35
30	1.90	60	0.70

Table A4.6 Station MMA7, Replicate 4 Mussel Measurements

Assigned Number	**Length (cm)**	**Assigned Number**	**Length (cm)**
1	1.00	31	2.55
2	1.30	32	1.10
3	0.95	33	2.10
4	1.60	34	1.60
5	1.65	35	1.35
6	1.95	36	1.15
7	1.05	37	2.05
8	1.25	38	2.15
9	1.70	39	4.95
10	1.75	40	1.40
11	1.50	41	1.85
12	1.15	42	0.35
13	1.20	43	1.00
14	1.70	44	1.60
15	1.75	45	1.65
16	1.25	46	1.80
17	1.00	47	1.30
18	1.40	48	1.55
19	1.40	49	1.95
20	1.55	50	2.95
21	1.35	51	1.65
22	0.10	52	1.95
23	0.95	53	1.15
24	3.15	54	1.80
25	1.80	55	2.50
26	1.25	56	2.00
27	1.20	57	1.05
28	2.05	58	1.15
29	1.30	59	1.85
30	0.80	60	1.90

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Assigned Number	Length (cm)
61	2.40
62	1.10
63	1.40
64	1.30
65	1.70
66	0.75
67	2.55
68	1.60
69	1.80
70	0.85
71	1.20
72	1.05
73	1.90
74	0.95
75	4.90
76	1.65
77	0.70
78	1.80
79	1.35
80	1.20
81	1.55
82	1.45
83	1.95
84	2.30
85	1.30
86	1.20
87	1.50
88	1.85
89	4.90
90	1.45

Assigned Number	Length (cm)
91	0.80
92	1.55
93	2.45