A BASELINE CHARACTERIZATION OF THE FAUNAL COMMUNITIES IN EELGRASS RESTORATION AREAS IN UPPER FRENCHMAN BAY

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This project was a collaborative effort of the summer 2013 members of the Community Environmental Health Laboratory and the Bangor High School group: Dr. Jane Disney, Dr. George Kidder; marine specialist Shannon White; intern Elizabeth Thompson, with assistance from interns Lukas Thorburn and Hanna Mogensen, phytoplankton monitor Ashley Heinze, special projects manager Duncan Bailey and education and outreach coordinator Jordan Bailey; and the Bangor High School group, Dr. Jennifer Page, Mr. Ted Taylor, Helen Zhang, and Aidan Coyne. David Clare, a PhD candidate studying marine ecology at the University of Liverpool, Helen Hess, of College of the Atlantic, Karen James, of MDIBL, and volunteers Genevieve Davis, Eliza Rockefeller, Paige LeDuc, and Grace Drennan also contributed to project efforts.

Dr. George Kidder is particularly acknowledged for his facilitation of travel to the field sites and for designing and creating some of the necessary sampling equipment. Our interns who were not specifically assigned to this project are acknowledged as Lukas Thorburn contributed to map-building and, along with Hanna Mogensen, helped with field-work and sample processing. Ashley Heinze conducted analysis of photos to determine percentage cover of mussels in restoration areas. Duncan Bailey helped us grapple with Microsoft Access and Jordan Bailey brought public exposure to our work by bringing a reporter out to observe our field sampling.

This project would not have been possible without the Bangor High School group whose members contributed a great deal of time and energy to facilitating travel to field sites, conducting field work, processing samples, identifying organisms, and supporting the project work even after their time at MDIBL was completed.

David Clare put in a large number of volunteer hours to help with organism ID. Helen Hess, who was supported through Karen James and the BioTrails project, also contributed to organism ID for this project. Karen James also offered to carry out DNA barcoding to assist with organism ID.

Gen Davis put many hours into quantifying organisms scraped from the larval collector plates. Eliza Rockefeller, Paige LeDuc, and Grace Drennan have also been involved with processing the larval collector scrapings.

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I. Purpose

The Community Environmental Health Laboratory (CEHL) at Mount Desert Biological Laboratory has carried out eelgrass restoration efforts in Upper Frenchman Bay since 2007. In an effort to understand how restored eelgrass functions as habitat in comparison with bare sediment, a study was launched in the summer of 2013 to make this comparison by examining the different faunal communities inside and outside of eelgrass habitat. In 2013, however, there was a widespread disappearance of eelgrass in the upper bay (Figure 1). Accordingly, baseline data was gathered in restoration areas where eelgrass had occurred historically in order to serve as a kind of "pre-restoration" proxy for community composition. In addition, these community data could be compared with data collected in areas of the bay where eelgrass did occur in 2013.

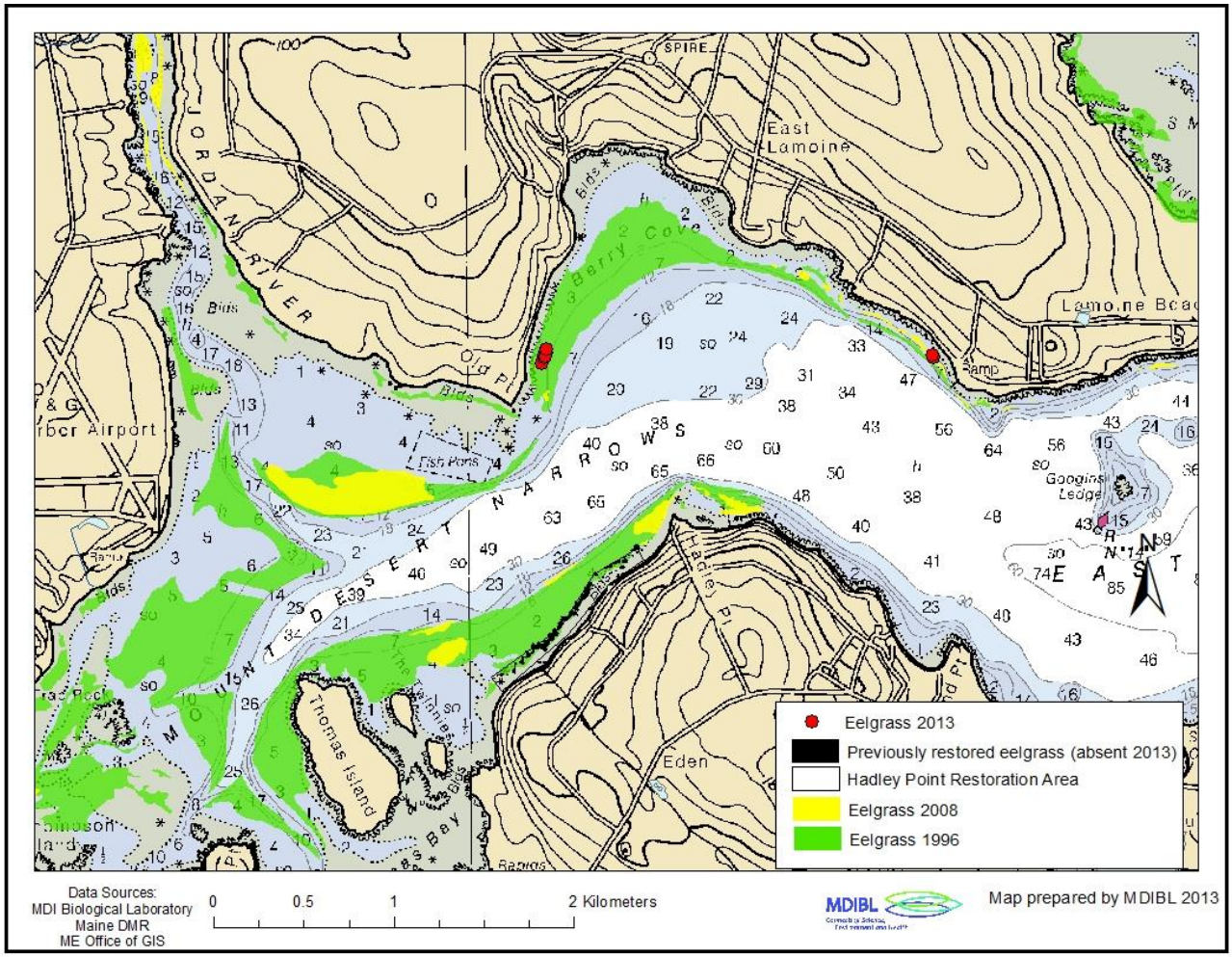


Figure 1. Historic and current documented coverage of eelgrass in upper Frenchman Bay.

II. Study Area

Hadley Point and Berry Cove are two locations where CEHL has historically carried out eelgrass restoration. We therefore established sampling areas at these sites (Figure 2). At Hadley Point, we divided the restoration area into three distinct sampling areas; Hadley Point 1, Hadley Point 2, and Hadley Point

3. At Berry Cove, we established one bare sediment sampling area. In 2013, eelgrass was present in a small area at the southern end of Berry Cove (area 5 for the nutrient experiment) and it was also present at Bar Island. Therefore, we conducted sampling in these areas in order to make some 2013 comparisons between bare sediment communities and communities in eelgrass.

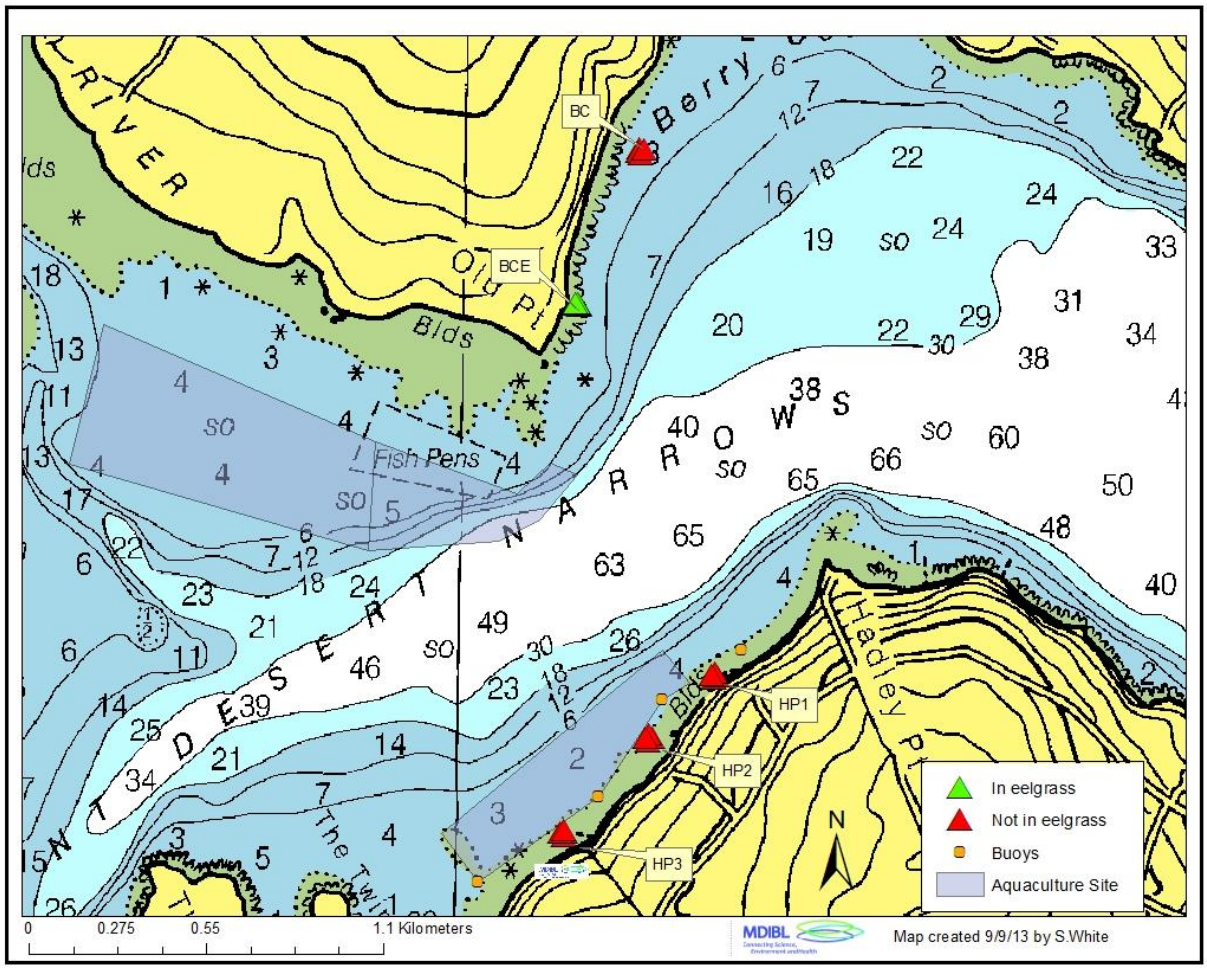


Figure 2. Berry Cove and Hadley Point sample sites for the 2013 survey of faunal communities in eelgrass and eelgrass restoration areas. Hadley Point 1-3 (HP1-3), Berry Cove (BC) and Berry Cove Eelgrass (BCE). Not shown is the sample site at Bar Island (BI).

III. Sampling Design and Sample Collection

Four sampling techniques were used to capture and characterize the different components of the faunal communities in the study areas. This included sampling the organisms living in the sediment (infauna), the organisms living on the sediment surface (epifauna), and organisms in the water column, including those in larval/juvenile life stages. Each sampling technique that was chosen needed to be replicable inside and outside of eelgrass. Community data is presented separately in the following sections for each sampling technique.

IV. Infauna

One group that we were interested in was the infaunal community, or the organisms that live in the sediment.

Field Sampling

Infauna were collected using a corer that was approximately 5cm in diameter and went 15cm into the sediment (Plate 1).

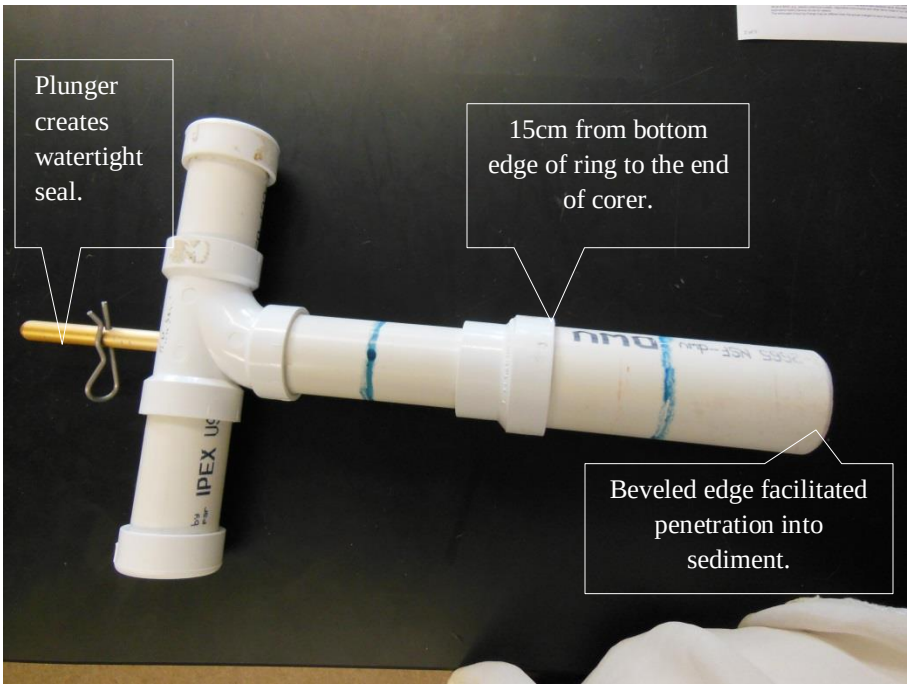


Plate 1. Infaunal corer used in 2013 survey of infauna at eelgrass and eelgrass restoration sites in Frenchman Bay. Corer created by Dr. George Kidder.

At our bare sediment "pre-restoration" sites, our sampling design within the restoration areas was ultimately based around an array of 12 larval collector poles that we deployed in each area at the beginning of the sampling season. These poles were deployed near the low water mark in the shallow subtidal to lower intertidal zone. The poles spanned a distance of 11-13.2m across shore and 8.7-13.75m from the shallowest to the deepest poles (Figure 3). The 6 yellow collector poles (marking the larval collectors that would remain in the field until the end of the season) were used as a point of reference for the collection of core samples. A total of 12 cores, 2 cores (A and B) per yellow pole, were collected within each sampling area. Two cores were taken from within a 60 x 60cm quadrat set directly adjacent to the pole or set a specified distance seaward or shoreward of the pole. With respect to the latter, in some cases cores were taken 1.2m shoreward or seaward of the pole in order to adjust for differences in the area covered by the poles at the different sites (i.e. to make the sampling areas more comparable). Thus the distance covered from the shallowest to the deepest core samples ranged from 10.5-11.35m (from the original distance covered by the poles of 8.7-13.75m). At the Berry Cove Eelgrass site, two cores (A and B) were taken from within the quadrat in each of the three eelgrass patches that were present, for a total of 6 cores. At Bar Island, 12 cores (6 pairs of A and B cores) were collected in the eelgrass beds in "blank"

patches. Each site that a core was taken from was completely surrounded by eelgrass, but the cores were taken at the very edge of that spot.

To use the core, the plunger was pushed all the way to the end of the corer and then the corer was placed at the sediment surface. The core was pushed into the sediment until the bottom of the white ring marking 15cm reached the sediment surface. The person using the core would then work their hand underneath the bottom of the core to ensure that no sediment was lost during extraction. Using the plunger, cores were pushed out of the corer into labeled bags. Seawater was added to each bag to cover the core. The samples were each placed in a cooler (stacking the samples was avoided where possible) and back at the lab the samples were moved into the fridge (again avoiding stacking).

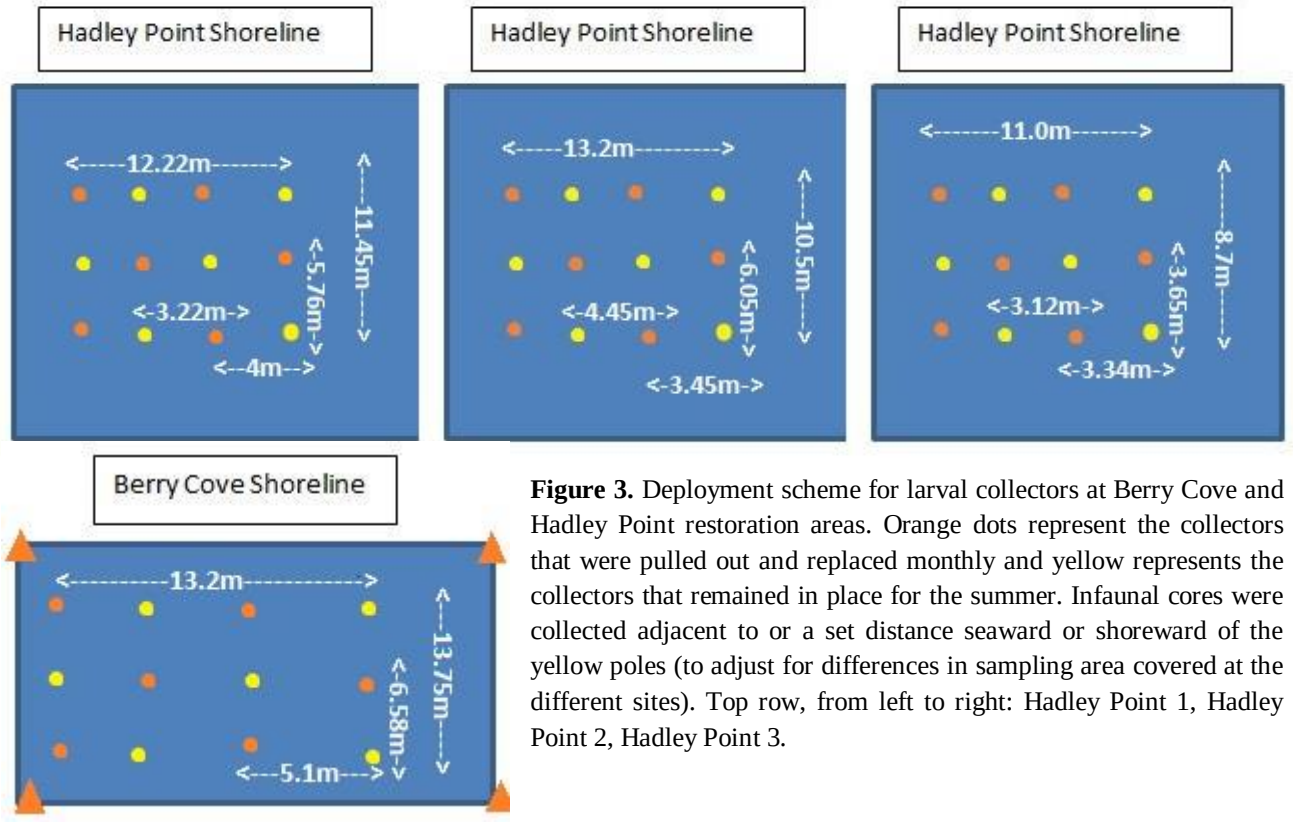


Figure 3. Deployment scheme for larval collectors at Berry Cove and Hadley Point restoration areas. Orange dots represent the collectors that were pulled out and replaced monthly and yellow represents the collectors that remained in place for the summer. Infaunal cores were collected adjacent to or a set distance seaward or shoreward of the yellow poles (to adjust for differences in sampling area covered at the different sites). Top row, from left to right: Hadley Point 1, Hadley Point 2, Hadley Point 3.

Sample Processing in the Lab

Samples were sieved using a 0.5mm mesh sieve in a tub with seawater. The sample in its entirety was poured into the sieve within the tub. The sample bag was rinsed with filtered seawater to get any of the remaining sediment from the sides and corners of the bag and this was poured onto the sieve with the rest of the sample. In order to minimize damage to the specimens in the sample, a gentle up and down motion was used for sieving, with the surface of the water kept close to the top of the sieve's walls. When the fine sediments were sieved from the sample, the remaining sample was poured into a large petri dish (pouring filtered seawater from behind the sieve as it is tipped over the petri dish helps to wash the sample into the dish). It was important to examine the sieve to make sure there were no specimens remaining intertwined

in the mesh. The sample in the petri dish was then jiggled to help level it out and the cloudy seawater was decanted into a separate dish (it was important to make sure that no organisms came out of the sample in the decanted water). Filtered seawater was then gently poured into the sample dish to help with sorting. The samples were sorted (from one end of the petri dish to the other) under dissecting microscopes. Specimens that were collected through the sorting process were preserved in 98% ethanol. These specimens were identified using Pollock (1998) and recorded on the datasheet for their respective core.

Plate 2. Sorting core samples under the dissecting microscope. Shannon White and Liz Thompson.

Data Analysis

Infaunal data were entered into a database using Microsoft Access. These data were exported to Microsoft Excel for analysis. Organism abundances were averaged for paired A and B cores at each site. This resulted in 6 replicates for all three Hadley Point sites and for Berry Cove and 3 replicates for Berry Cove Eelgrass. Not all samples were processed from the Bar, which resulted in 4 replicates, with one replicate represented by a single core. The Shannon-Wiener diversity index, species richness (i.e. total number of taxa), and total individuals per sample were calculated and compared among sites. Prior to calculating the Shannon-Wiener index and species richness, individuals that were identified to a lower taxonomic resolution (polychaete unid, Lumbrineridae unid, Spionidae unid, and Nereididae unid) were removed. There were also individuals that were only identified to Nemertea unid and Maldanidae unid, but these groups were left in for analysis because individuals represented by these names did not occur in the same samples as individuals identified to a higher taxonomic resolution in the same group, thus eliminating the possibility of counting one species as two separate taxa. All statistical analyses were conducted using Microsoft Excel and R. A Kruskal-Wallis test was used to compare diversity indices among sites as the data did not all satisfy the assumptions of homogeneity of variance and normality necessary to conduct ANOVA.

Results

Kruskal-Wallis showed no significant differences in Shannon-Wiener index among sites (χ^2= 4.42, df=5, p=0.49) (Figure 4A), in species richness among sites (χ^2= 4.54, df=5, p=0.475) (Figure 4B), in total number of individuals per sample among sites (mussel seed included) (χ^2= 6.15, df=5, p=0.291) (Figure 5A), and no significant difference in total number of individuals per sample among sites (mussel seed excluded) (χ^2= 4.21, df=5, p=0.519) (Figure 5B). While the differences were not significant, Berry Cove and Hadley Point 2 and 3 had the highest Shannon-Wiener indices and species richness, while the high number of mussel seeds associated with eelgrass in a sample from Berry Cove Eelgrass contributed to the very high number of individuals recorded for this site (Plate 3). When mussel seed was excluded, Hadley Point 3 and Berry Cove had the highest average number of individuals per sample. While the mussel seed associated with the eelgrass blades and filamentous structures in the Berry Cove Eelgrass sample is not

representative of the infaunal community, this does exhibit the function of eelgrass structures as habitat for mussels. Hadley Point 3 had relatively high values for each of the diversity indices. This was the shallowest of the three Hadley Point sites and the sediment felt sandier than in the other sites (as observed in the field), which may or may not have contributed to the differences observed. Table 1 depicts a list of each of the taxa recorded from the samples collected during the infaunal survey and Table 2 depicts average number of individuals per sample (1 sample is the average of a pair of A and B cores).

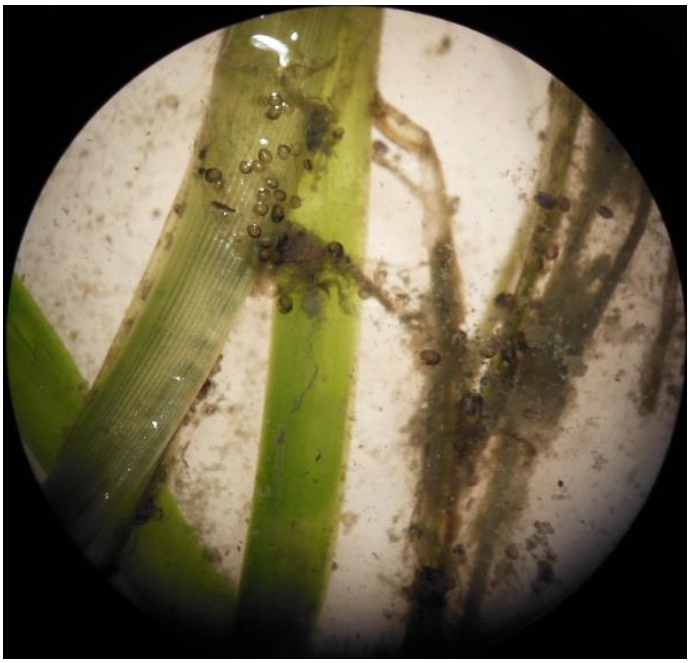


Plate 3. Mussel seed associated with eelgrass from Berry Cove.

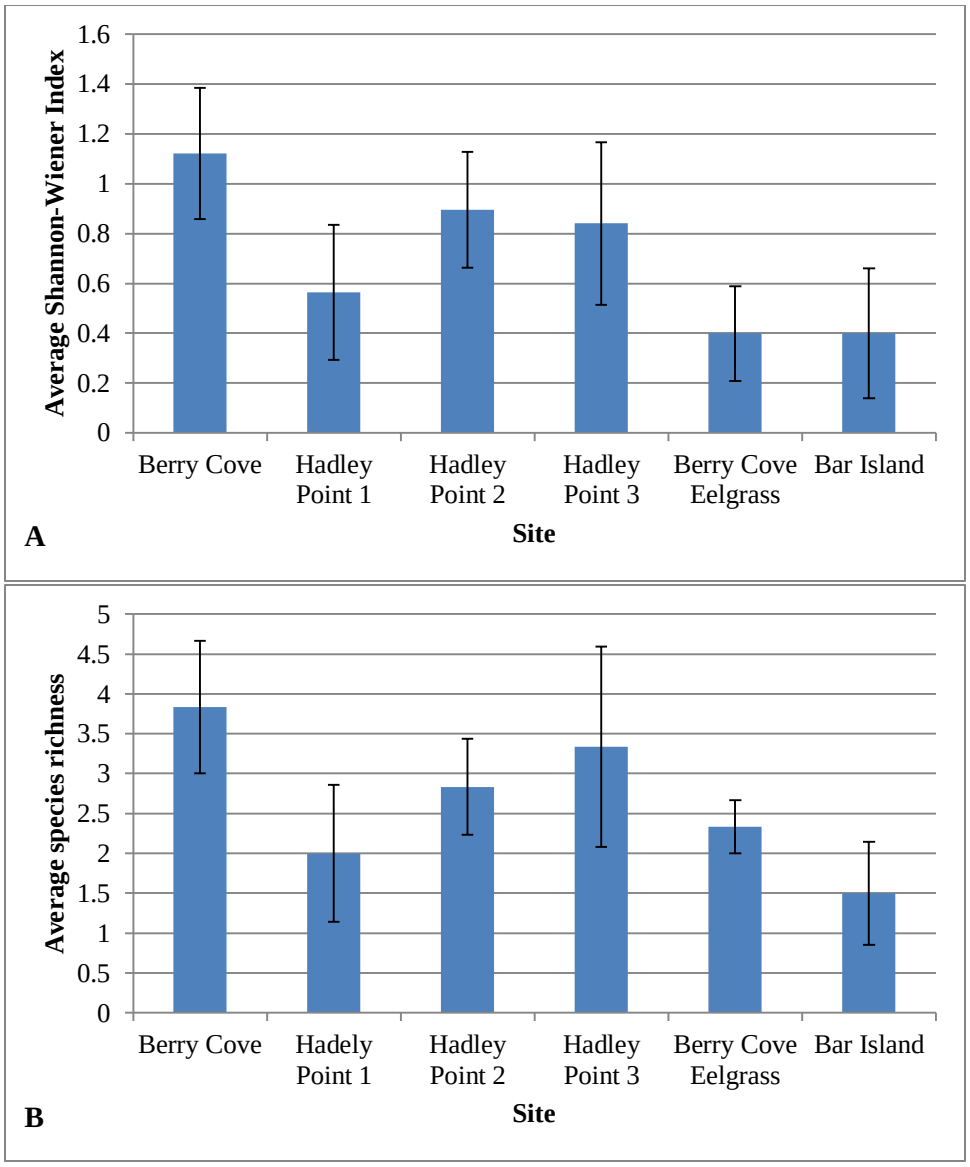


Figure 4. A) Average Shannon-Wiener index B) average species richness per sample by site for infaunal organisms collected in cores that were ~5cm in diameter and went 15cm into the sediment. One sample is the average of two cores, with the exception of one sample from Bar Island that is represented by a single core. Berry Cove Eelgrass and Bar Island are eelgrass sites and the remaining sites were bare sediment in eelgrass restoration areas. Error bars are standard error. Berry Cove and Hadley Point 1-3 (n=6), Berry Cove Eelgrass (n=3), Bar Island (n=4).

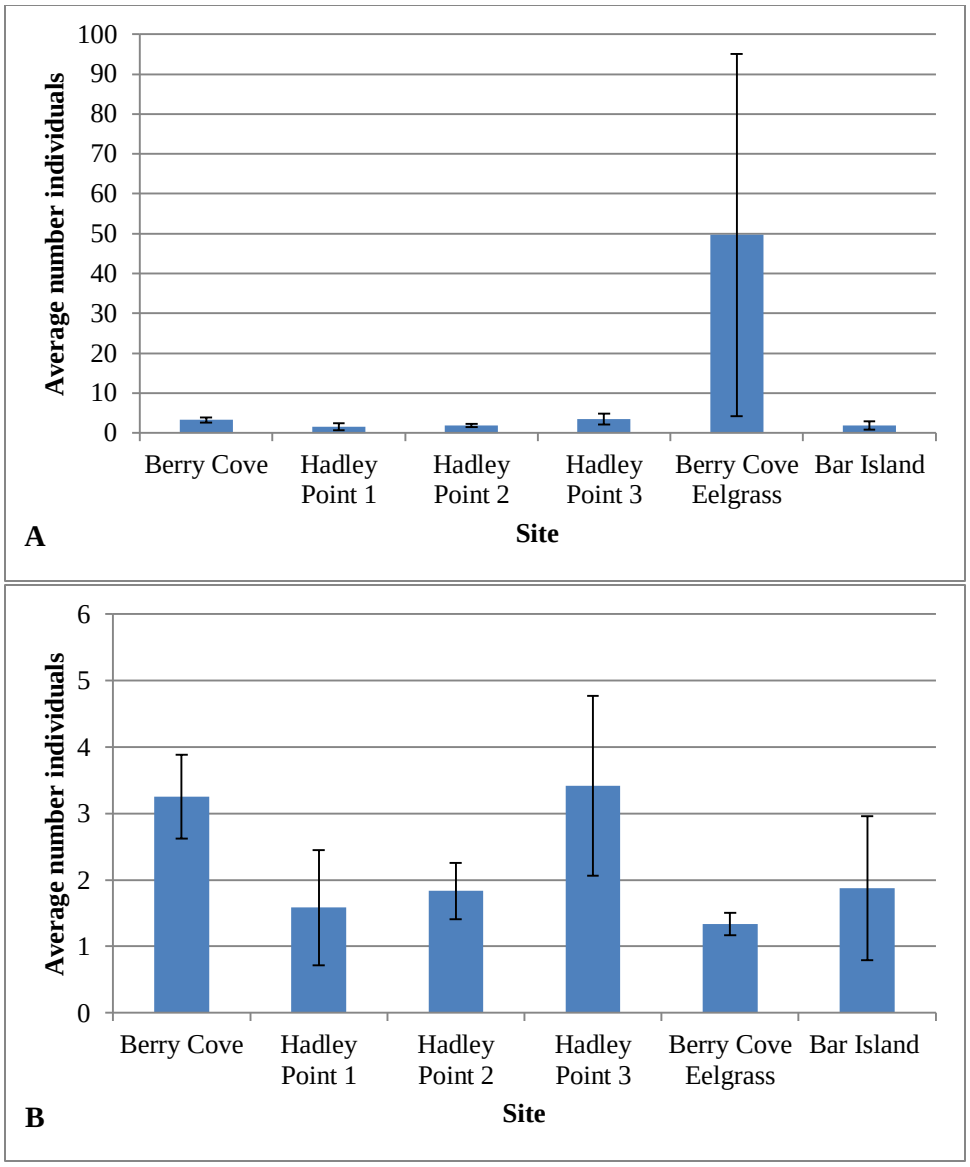


Figure 5. Average number of individuals per sample by site for infaunal organisms collected in cores that were ~5cm in diameter and went 15cm into the sediment. One sample is the average of two cores, with the exception of one sample from Bar Island that is represented by a single core. A) Mussel seed included B) mussel seed excluded; a very high number of mussel seeds were associated with eelgrass material in one of the Berry Cove Eelgrass core samples. Berry Cove Eelgrass and Bar Island are eelgrass sites and the remaining sites were bare sediment in eelgrass restoration areas. Error bars are standard error. Berry Cove and Hadley Point 1-3 (n=6), Berry Cove Eelgrass (n=3), Bar Island (n=4).

Table 1. List of the infaunal taxa identified during a survey of eelgrass restoration areas and areas where eelgrass was present in Frenchman Bay in 2013. The column 'taxon identified' represents the highest taxonomic resolution achieved in the identification of each taxon. Presence at each site denoted by x for Berry Cove (BC), Hadley Point 1-3 (HP1-3), Berry Cove Eelgrass (BCE), and Bar Island (BI).

Phylum	Class	Family	Taxon identified	BC	HP1	HP2	HP3	BCE	BI
Arthropoda	Malacostraca	Ampeliscidae	*Ampelisca macrocephala*			x			
Arthropoda	Malacostraca	Ampeliscidae	*Ampelisca vadorum*			x			
Arthropoda	Malacostraca	Ampeliscidae	*Ampelisca verrilli*	x					
Annelida	Polychaeta	Arenicolidae	*Arenicola* spp.						x
Nemertea	Anopla	Lineidae	*Cerebratulus lacteus*	x					
Annelida	Clitellata	Tubificidae	*Clitellio arenarius*	x	x	x	x		x
Arthropoda	Maxillopoda		Copepoda unid					x	
Annelida	Polychaeta	Phyllodocidae	*Eteone* sp.					x	
Annelida	Polychaeta	Maldanidae	*Euclymene zonalis*				x		
Annelida	Polychaeta	Glyceridae	*Glycera dibranchiata*				x		
Annelida	Polychaeta	Nereididae	*Hediste diversicolor*					x	
Annelida	Polychaeta	Polynoidae	*Lepidonotus squamatus*				x		
Mollusca	Gastropoda	Littorinidae	*Littorina littorea*						x
Annelida	Polychaeta	Lumbrineridae	Lumbrineridae unid.		x				
Annelida	Polychaeta	Maldanidae	Maldanidae unid.			x			
Mollusca	Bivalvia	Myidae	*Mya arenaria*				x		
Arthropoda	Malacostraca	Mysidae	*Mysis stenolepis*		x				
Mollusca	Bivalvia	Mytilidae	*Mytilus edulis* seed					x	
Nematoda			Nematodes		x		x	x	x
Nemertea			Nemertea unid.			x	x		
Annelida	Polychaeta	Nephtyidae	*Nephtys caeca*		x				
Annelida	Polychaeta	Nereididae	Nereididae unid.					x	
Annelida	Polychaeta	Nereididae	*Nereis pelagica*	x	x	x	x		x
Annelida	Polychaeta	Lumbrineridae	*Ninoe nigripes*	x	x	x	x		
Annelida	Polychaeta	Pectinariidae	*Pectinaria gouldii*	x					
Annelida	Polychaeta	Flabelligeridae	*Pherusa plumosa*				x		
Annelida	Polychaeta		Polychaete unid.			x	x	x	
Annelida	Polychaeta	Spionidae	*Polydora cornuta*	x	x	x	x		
Annelida	Polychaeta	Spionidae	*Prionospio heterobranchia*	x	x	x	x		
Annelida	Polychaeta	Lumbrineridae	*Scoletoma acicularum*				x		
Annelida	Polychaeta	Lumbrineridae	*Scoletoma fragilis*			x			
Annelida	Polychaeta	Spionidae	*Spio setosa*	x	x				
Annelida	Polychaeta	Spionidae	Spionidae unid.			x			
Annelida	Polychaeta	Spionidae	*Spiophanes bombyx*	x				x	
Annelida	Clitellata	Tubificidae	*Tubificoides benedii*	x			x		
Tracheophyta	Liliopsida	Zosteraceae	*Zostera marina* seed			x	x	x	

Table 2. Average abundance per sample (1 sample = average of pair of A and B cores) per site of the infaunal taxa identified during a survey of eelgrass restoration areas and areas where eelgrass was present in Frenchman Bay in 2013. The column 'taxon identified' represents the highest taxonomic resolution achieved in the identification of each taxon. The presence of nematodes and *Z. marina* seeds/cases is denoted by "P." The absence of *Z. marina* seeds may or may not be a reflection of different sample processors including or excluding them in the sample during processing. Berry Cove and Hadley Point 1-3 (n=6), Berry Cove Eelgrass (n=3), Bar Island (n=4).

Phylum	Class	Family	Taxon identified	BC	HP1	HP2	HP3	BCE	BI
Arthropoda	Malacostraca	Ampeliscidae	*Ampelisca macrocephala*			0.333			
Arthropoda	Malacostraca	Ampeliscidae	*Ampelisca vadorum*			0.083			
Arthropoda	Malacostraca	Ampeliscidae	*Ampelisca verrilli*	0.083					
Annelida	Polychaeta	Arenicolidae	*Arenicola* spp.						0.125
Nemertea	Anopla	Lineidae	*Cerebratulus lacteus*	0.250					
Annelida	Clitellata	Tubificidae	*Clitellio arenarius*	0.167	0.083	0.083	0.750		1.375
Arthropoda	Maxillopoda		Copepoda unid					0.333	
Annelida	Polychaeta	Phyllodocidae	*Eteone* sp.					0.167	
Annelida	Polychaeta	Maldanidae	*Euclymene zonalis*				0.083		
Annelida	Polychaeta	Glyceridae	*Glycera dibranchiata*				0.083		
Annelida	Polychaeta	Nereididae	*Hediste diversicolor*					0.167	
Annelida	Polychaeta	Polynoidae	*Lepidonotus squamatus*				0.083		
Mollusca	Gastropoda	Littorinidae	*Littorina littorea*						0.250
Annelida	Polychaeta	Lumbrineridae	Lumbrineridae unid.		0.083				
Annelida	Polychaeta	Maldanidae	Maldanidae unid.			0.083			
Mollusca	Bivalvia	Myidae	*Mya arenaria*				0.333		
Arthropoda	Malacostraca	Mysidae	*Mysis stenolepis*		0.167				
Mollusca	Bivalvia	Mytilidae	*Mytilus edulis* seed					48.333	
Nematoda			Nematodes		P		P	P	P
Nemertea			Nemertea unid.			0.167	0.167		
Annelida	Polychaeta	Nephtyidae	*Nephtys caeca*		0.083				
Annelida	Polychaeta	Nereididae	Nereididae unid.					0.167	
Annelida	Polychaeta	Nereididae	*Nereis pelagica*	0.250	0.583	0.167	0.500		0.125
Annelida	Polychaeta	Lumbrineridae	*Ninoe nigripes*	0.250	0.083	0.333	0.333		
Annelida	Polychaeta	Pectinariidae	*Pectinaria gouldii*	0.083					
Annelida	Polychaeta	Flabelligeridae	*Pherusa plumosa*				0.083		
Annelida	Polychaeta		Polychaete unid.			0.167	0.083	0.333	
Annelida	Polychaeta	Spionidae	*Polydora cornuta*	0.750	0.167	0.083	0.333		
Annelida	Polychaeta	Spionidae	*Prionospio heterobranchia*	1.167	0.167	0.167	0.333		
Annelida	Polychaeta	Lumbrineridae	*Scoletoma acicularum*				0.083		
Annelida	Polychaeta	Lumbrineridae	*Scoletoma fragilis*			0.083			
Annelida	Polychaeta	Spionidae	*Spio setosa*	0.083	0.167				
Annelida	Polychaeta	Spionidae	Spionidae unid.			0.083			
Annelida	Polychaeta	Spionidae	*Spiophanes bombyx*	0.083				0.167	
Annelida	Clitellata	Tubificidae	*Tubificoides benedii*	0.083			0.167		
Tracheophyta	Liliopsida	Zosteraceae	Zostera marina seed/case			P	P	P	

V. Larval Collectors

Eelgrass habitat adds structural complexity to the environment in which it occurs and provides a place of settlement and attachment for other organisms, including larval forms floating in the water column. As such, we wanted to be able to make comparisons of settlement inside and outside of eelgrass habitat. To do this we utilized larval collectors, which introduced a settlement surface that could be placed both inside and outside of eelgrass. We were also interested in differences in the organisms that settle at different points during the summer, so we left half of the collectors in for the duration of the sampling season, while the other half's plates were replaced part of the way through the summer.

Field Sampling

Larval collectors (Plate 4A) each consisted of a 10cm x 15cm PVC plate that was roughened on one side. Each plate was attached with two zip ties to a 5ft PVC pole. Two holes were drilled into each pole. One hole was located 30cm from the bottom to mark the depth to which the pole should be hammered into the sediment and the second hole was drilled 45cm from the bottom of the pole to mark the place of attachment for the PVC plate, which would be positioned to sit 15cm above the sediment surface. The top of each pole was spray painted either yellow or orange to indicate which plates needed to be replaced part of the way through the sampling season.

The collector poles were hammered 30cm into the sediment so that the PVC plate sat 15cm above the sediment (Plate 4B). The plates were oriented so that they pointed seaward. On June 11, they were

Plate 4. A) Larval collectors. B) Shannon White and Liz Thompson deploy larval collectors by hammering them into the sediment.

deployed in an alternating pattern of orange and yellow poles in three rows of four in the shallow subtidal/lower intertidal zone (Figure 6). Yellow poles marked the larval collectors that would stay in for the duration of the sampling season while the orange poles marked the collectors with plates that would be replaced part of the way through the sampling season.

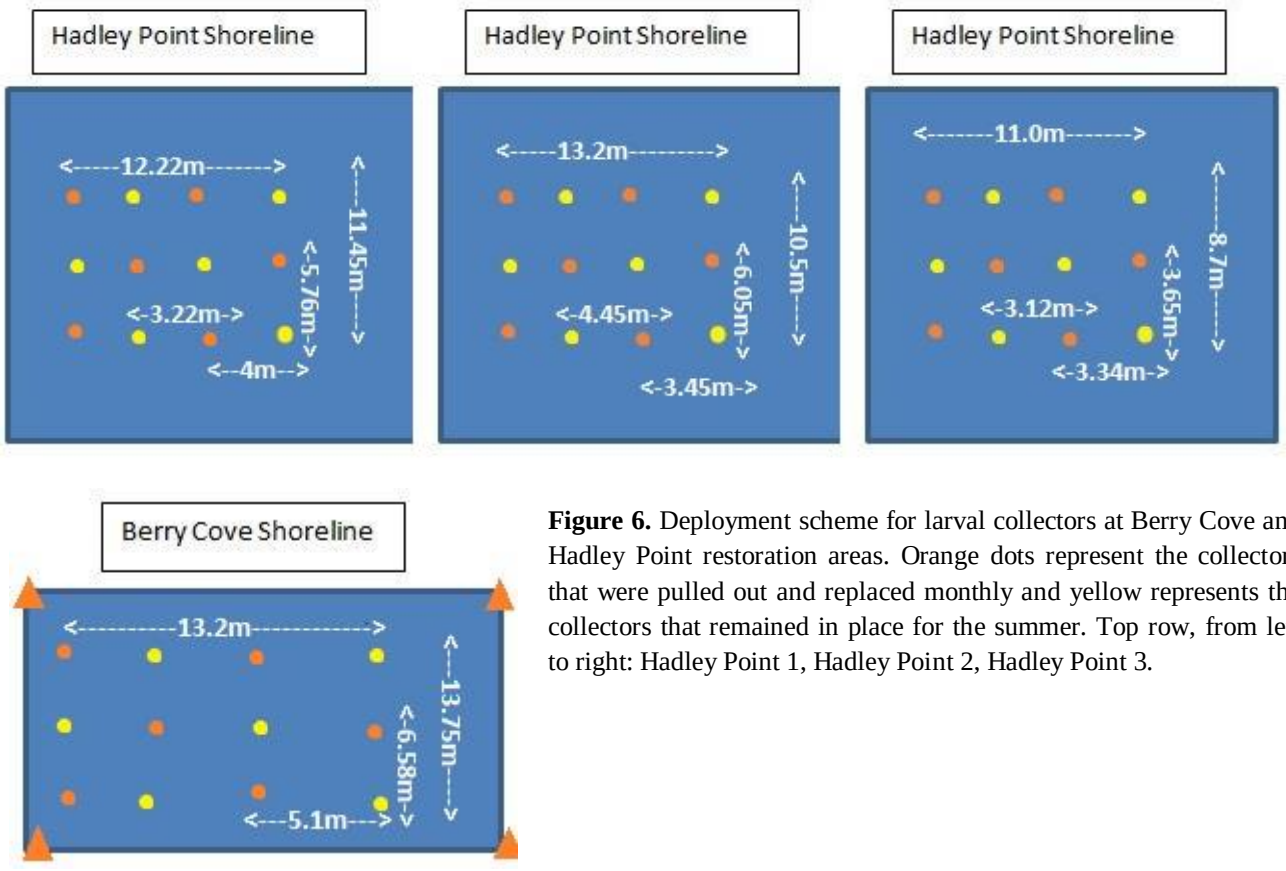


Figure 6. Deployment scheme for larval collectors at Berry Cove and Hadley Point restoration areas. Orange dots represent the collectors that were pulled out and replaced monthly and yellow represents the collectors that remained in place for the summer. Top row, from left to right: Hadley Point 1, Hadley Point 2, Hadley Point 3.

On July 9 and 10, the plates on the collectors marked by orange poles were replaced with fresh plates. The zip ties holding the plates on the poles were snipped (Plate 5) and the plates were placed in labeled plastic containers that had been spritzed with filtered seawater. These containers were then placed in a cooler. New plates were reattached to the poles and the larval collectors were redeployed (Plate 6).

At Hadley Point, all 12 poles (orange and yellow) were redeployed in a new configuration of two rows of six poles, each set seaward of the original array.

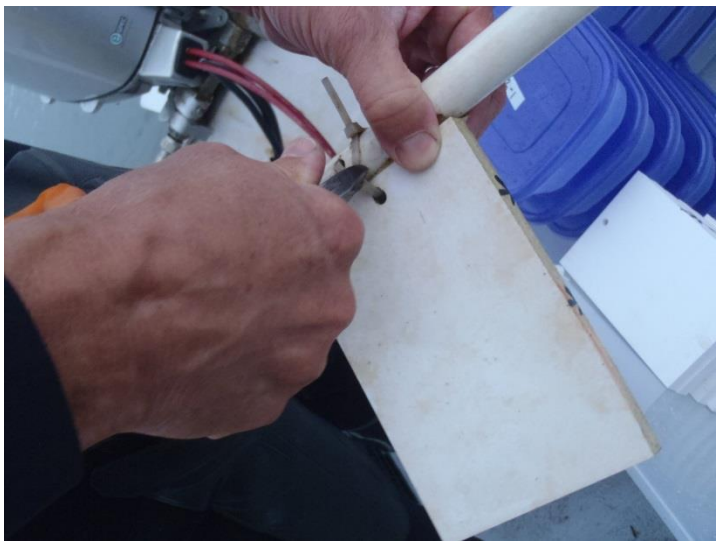


Plate 5. A sample plate is collected by snipping the zip ties which attached it to the PVC pole.

Poles were redeployed in this configuration in order to avoid exposure of the plates to air (and dessication) at extreme low tides (we had observed the plates out of water). At Hadley Point 2 and 3, the deepest row of poles in the new configuration was 15m seaward from the deepest row of poles in the original array. At Hadley Point 1 the deepest row of poles was only 10m seaward from the deepest row of poles in the original array, as this was our deepest Hadley Point area. The poles were spaced 5m apart from each other within a row and the two rows were also 5m apart. The poles at Berry Cove were not exposed to air in their original configuration and were left in the three rows of four poles.

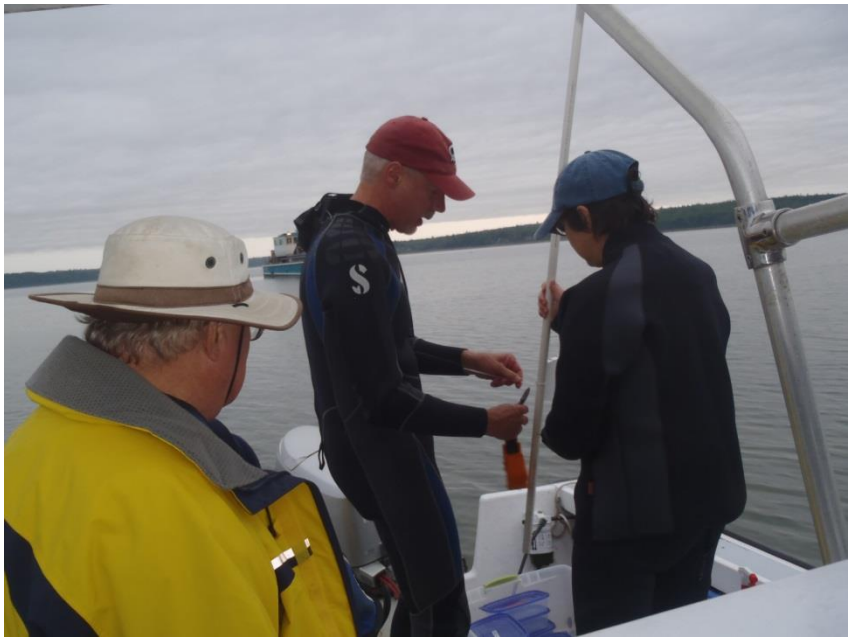


Plate 6. Ted Taylor and Dr. Jane Disney reattach a larval collector plate to the pole for redeployment.

In addition to setting up larval collectors at Hadley Point and Berry Cove in bare sediment, collectors were set up in eelgrass areas at Berry Cove and at the Bar. On July 10, six larval collectors were deployed at Berry Cove in three eelgrass patches (two collectors in each patch). On July 12, five larval collectors were redeployed at the Bar with new plates. Six collectors had originally been deployed earlier in the summer, but these were not located *in* the eelgrass and they were also exposed to air at low tide.

On August 7 and August 8, all of the larval collector plates and poles were retrieved and the plates were placed in coolers to be brought back to the lab for processing.

Sample processing in the lab

In the lab, plates were removed from their plastic containers and larger organisms were picked off with forceps and preserved in labeled tubes with 80% ethanol. Periwinkles were recorded on the datasheet and were set free. Where present, a subsample of the hydroids attached to the plates was picked off the plates and preserved. Therefore, some of the hydroids may have remained in the algal masses that were associated with many of the plates. To collect the remainder of the organic material on the plates, the front, back and edges of each plate were scraped into the container the plates were collected in in the field. A butter knife was aligned with the top edge of the plate (nearest to the hole which connects the plate to the pole). One person sprayed the plate just under the knife with two sprays of filtered seawater

and the knife was used to scrape part way down the plate. This was repeated two more times for a total of six sprays and three scrapes down the long surface of the plate. This was typically followed by one additional spray and one long scrape down the whole plate. The knife was rinsed into the container as needed. This was carried out for both sides of the plate. Both sides were also wiped downwards with a finger after scraping with the knife. The finger was also rinsed into the container as necessary. All edges of the plate were also scraped with the knife and wiped with a finger (both rinsed into the container as necessary). Ultimately, we tried to get as much of the visible material off the plates as possible using the knife and wiping with the finger, while minimizing the amount of seawater sprayed. See Plates 7 and 8 for examples of organisms on larval collector plates prior to scraping.

*The plate scrapings from the samples collected in July were only from the roughened side of the plate as opposed to those collected in August which had all sides and edges of the plates scraped.

The slurry remaining in the container was pipetted into a tube or a larger sample container and fixed with Lugol's iodine (a drop of iodine for every 2ml of liquid in the sample). Samples were then stored in the dark. The samples collected in August were stored in the fridge because of the high algal content of many of them (it did not seem like the Lugol's was adequately preserving them). Ultimately, all of the larval plate samples were stored the fridge.

Plate scrapings have/will be analyzed by pipetting a 1ml subsample from the container fixed with Lugol's onto a Sedgewick Rafter slide. Organisms observed on the slide are quantified and abundance is multiplied up for the original volume of the container, though starting volumes varied depending on the amount of seawater used to spray the plate during the scraping process.

Plate 7. Above: *Asterias* spp. attached to face of collector plate. Below: Scale worm attached to edge of collector plate.

*Amphipods (preserved in the large organism tubes) were very challenging to try to identify under the dissecting scope. Representatives of the amphipod types observed were provided to Karen James for DNA barcoding in order to reach an accurate identification (one shrimp and a barnacle sample were also provided). Unidentified amphipods are currently referred to as types A-G and their identities can be updated in the Access Database for "Collectors - Large Organisms" on the CEHL drive.

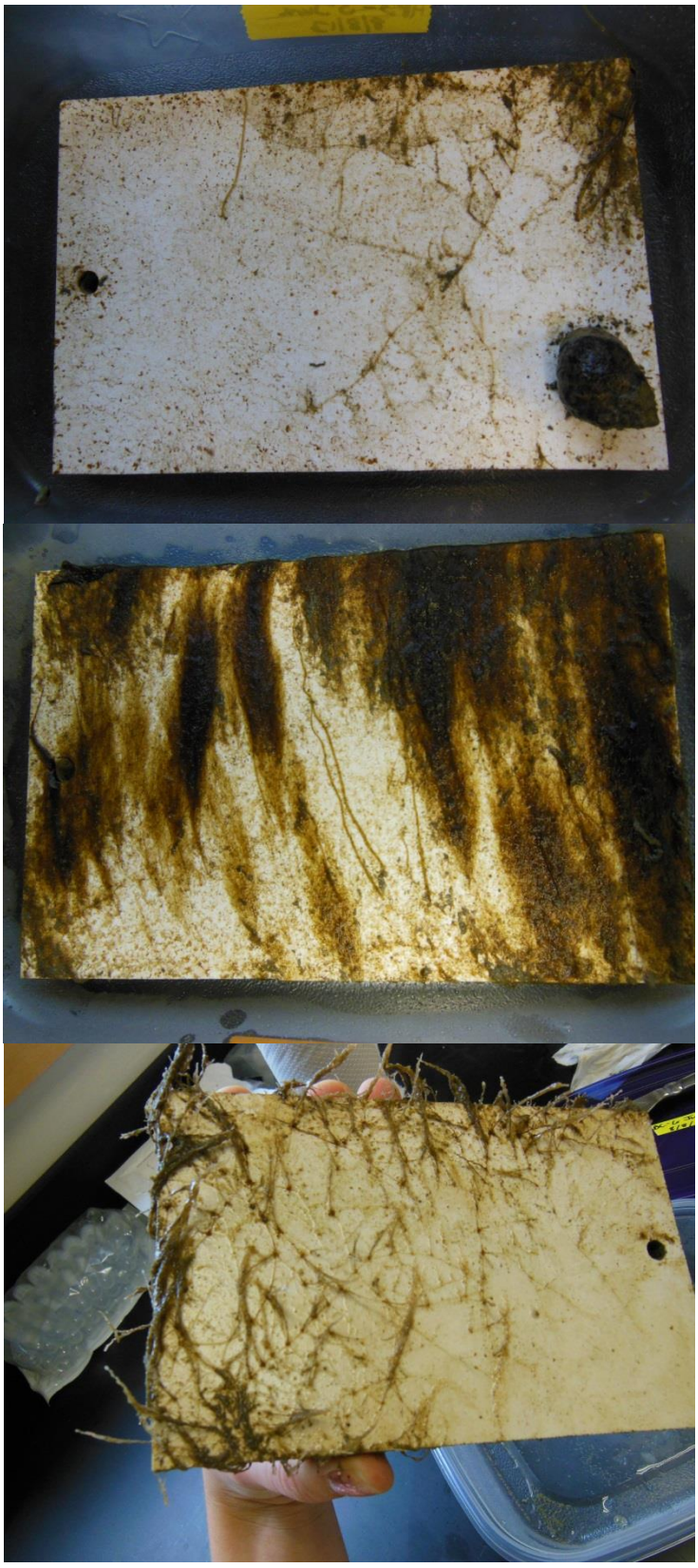


Plate 8. Top: *Littorina littorea;* middle: heavy algal cover on plate; bottom: hydroids prevalent on collector plate.

Data analysis

Data were entered into a Microsoft Access database and were exported to Microsoft Excel. Data were analyzed in Microsoft Excel in order to produce graphs and tables depicting the presence/absence and abundance of taxa, the average number of taxa, and the average total number of individuals represented by the "larger" organisms and associated smaller organisms on the larval collector plates. In determining the number of taxa and number of individuals per plate, algae were excluded from both. Hydroids were not recorded as individuals and were therefore not included in counts of individuals per plate. Gastropod unid, Mollusca unid, and polychaete unid were excluded from taxa number in order to avoid potentially double counting representatives of one taxon as multiple taxa. Amphipods referred to as types "A" and "AM" (most likely the male of type "A") were counted as the same taxon, while the rest of the amphipods were considered separate species, although there is a possibility for overlap.

Results

It important to note that the following data are representative only of what was collected and preserved in ethanol and are not representative of all taxa and total number of individuals from the plates, as a portion of the sample was scraped and preserved in Lugol's. While it was intended that only larger organisms would be collected from the plates and preserved in ethanol, it is clear that smaller organisms, such as copepods, very small mussel seed, very small barnacles, and the nudibranch *Tergipes tergipes* (found specifically on the campanularian hydroids), were collected in association with the larger organisms, particularly with hydroids or algal material. In addition to their presence in the sample, the numbers of the smaller organisms that were counted from the ethanol tubes would have been dependent on the *amount* of hydroid or algae that was present in the sample and how much of this was subsampled and preserved. Thus the amount of hydroid that was subsampled could have influenced organism numbers. We were primarily interested in preserving animals and therefore algae are better represented in the plate scraping sample tubes. The absence of algae in the following tables does not mean that algae wasn't on the plate (e.g. BCE-3A was the first plate with notable algal cover and HP2-4 June was also recorded during processing as having a lot of algae), but it reflects only what was counted in the ethanol tube.

Figure 7 depicts the averages of the total number of taxa and the total number of individuals represented by the "larger" organisms and associated smaller organisms that were collected from the plates that remained in the field from July to August. For plates that did not have hydroids or other large organisms on them, and therefore had no specimens preserved in ethanol, there was no chance of counting the smaller organisms associated with the plate or found in association with the larger organisms. Therefore, the lower abundances, taxa, and number of individuals at Bar Island and Berry Cove Eelgrass can partially be attributed to having some plates completely devoid of larger organisms ("empty" large organism tubes) and plates devoid of hydroids.

Some possible explanations for why hydroids were not found on the plates at the Bar could include differences in the hydrodynamics in the tidepool at the Bar compared with the other more open sites, shading out or other influences by eelgrass blades, preferential settlement on eelgrass blades or the kelp in surrounding area, possible exposure to air at low tide in the shallow tide pool which might cause unfavorable conditions for growth, and/or differences in temperature. Similarly, plates in eelgrass at Berry Cove (BCE) may have had few to no larger organisms because of shading out or other influences from the eelgrass blades, preferential settlement on eelgrass rather than on the plate, or because of other

environmental conditions at this site. With respect to the latter, clearly there were environmental differences between BCE and the other end of Berry Cove where eelgrass did not come up this season. Ideally, more collectors would have been deployed in eelgrass areas to make more accurate characterizations of organism colonization on the plates in eelgrass and better comparisons with colonization in bare sediment habitats.

Ultimately, at the bare sediment sites, we introduced a hard substrate that would otherwise not exist in these areas, creating a place of settlement. In the eelgrass sites, we introduced a hard substrate among the existing natural eelgrass habitat, so organisms that would typically settle in eelgrass probably favored the eelgrass over the hard plate. Because we introduced a hard substrate, it is possible that the organisms we collected are not representative of the organisms that would typically settle in an eelgrass bed. It would be interesting to make comparisons between the plates and actual eelgrass blades (perhaps the BioTrails work could be informative here). Still, the data presented here provide a baseline for what is living in the eelgrass restoration areas and the larval collector serves as a consistent unit of measure for comparing settlement inside and outside of eelgrass.

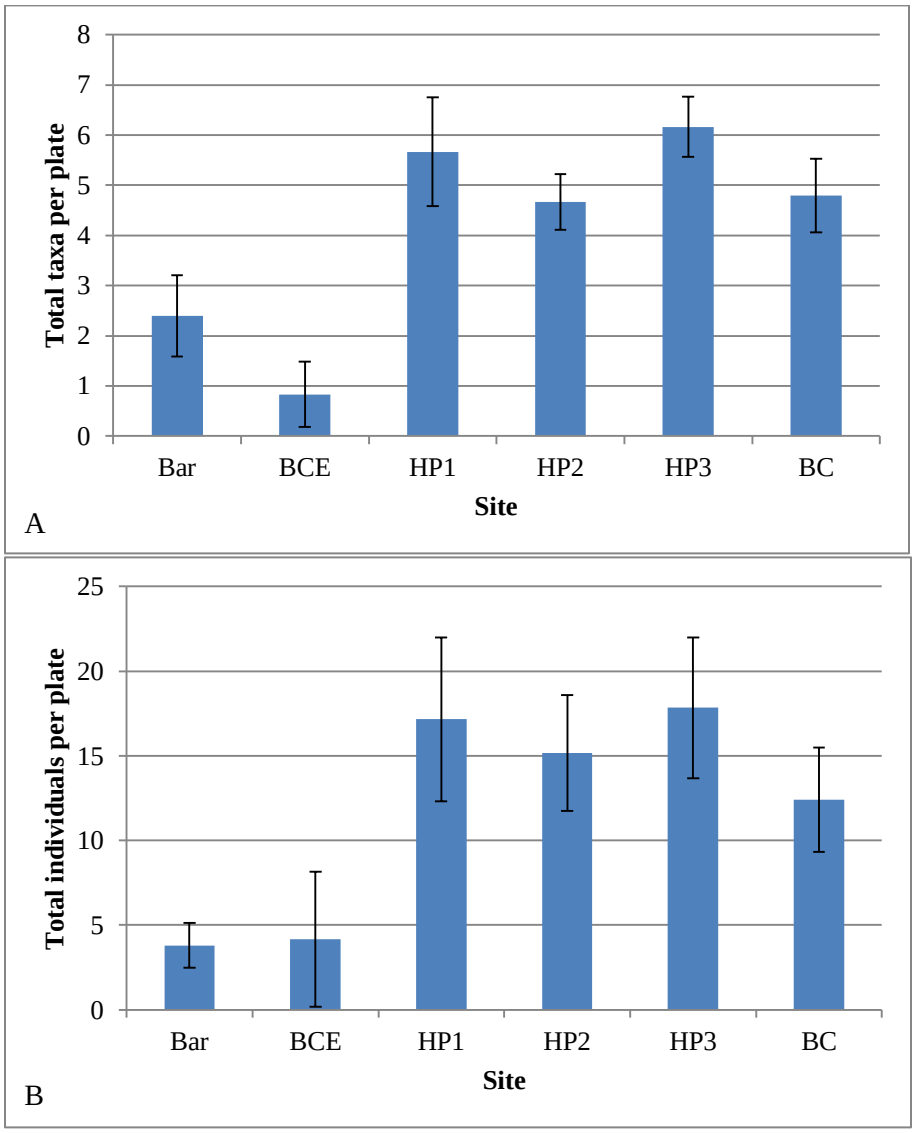


Figure 7. A) Average of the total number of taxa B) average of the total number of individuals representing only the "larger organisms" and associated smaller organisms that were present on the larval collector plates that remained in the field from July to August. Bar (n=5), BCE-Berry Cove Eelgrass (n=6), HP-Hadley Point sites 1-3 (n=6), BC-Berry Cove (n=5). Error bars are standard error.

Figure 8 depicts the averages of the total number of taxa and the total number of individuals represented by the larger organisms and associated smaller organisms that were collected from the plates that were in the field either from June to August or from July to August. In comparing plates that remained in the field for different durations, there did not seem to be any consistent differences in the total number of taxa that were recorded on the plates or the total number of individuals (both representative only of what was preserved in ethanol and does not include taxa and total individuals found in the scrapings that were fixed in Lugol's iodine). The very high number of total individuals recorded for Berry Cove in June can be attributed to a high number of mussel seed and barnacles. Hydroids were present both on the June and July plates at Berry Cove, which very small mussels and very small barnacles that otherwise would not have been collected in the "larger organism" tube were likely associated with. It is most likely that during sample processing for Berry Cove, large amounts of hydroid were subsampled initially, and the greater the amount of hydroid material, the higher number of smaller organisms there was likely to be in the sample.

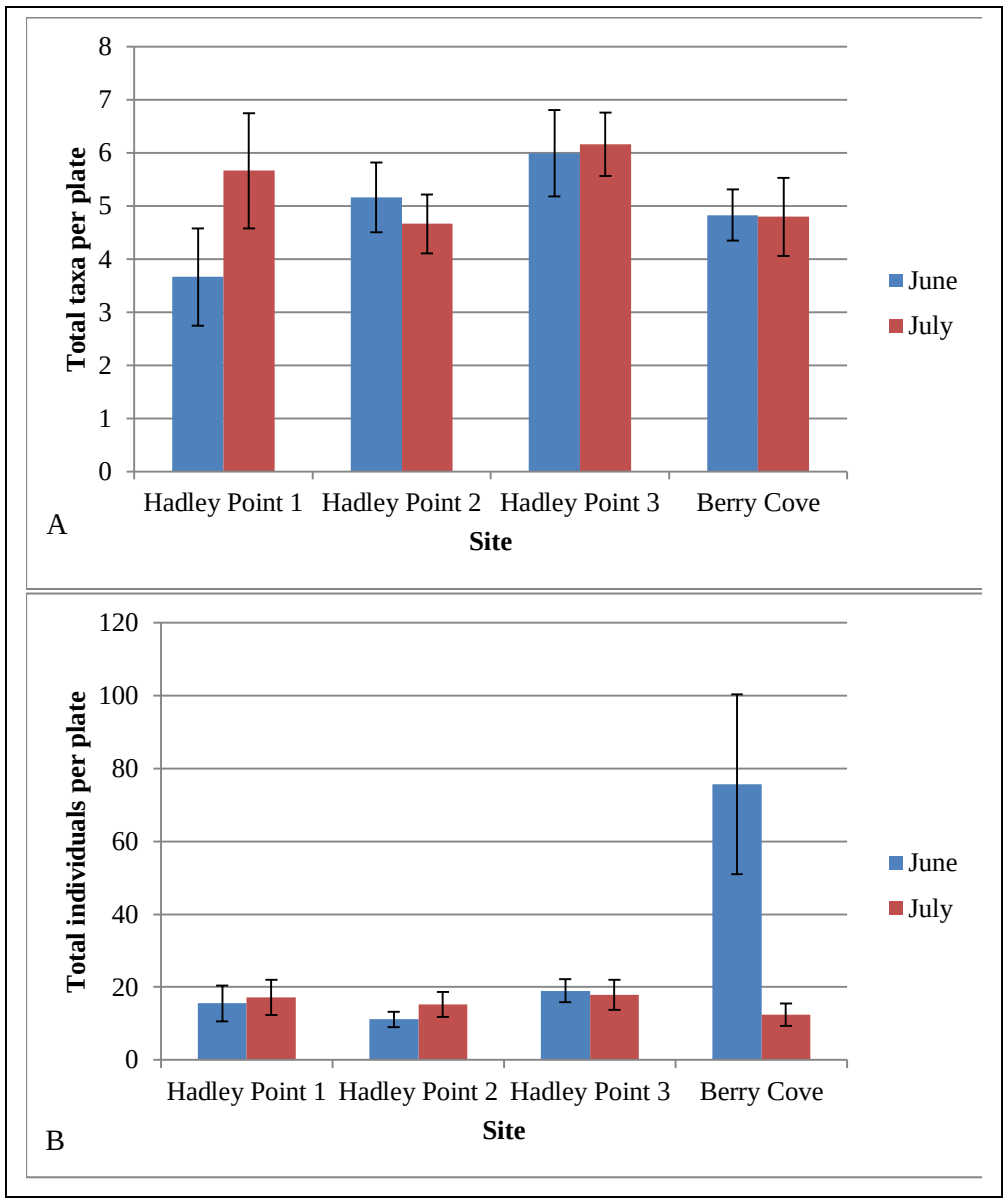


Figure 8. A) Average of the total number of taxa B) average of the total number of individuals representing only the "larger organisms" and associated smaller organisms that were present on the larval collector plates. "June" plates were left in the field from June to August and "July" plates were in the field from July to August. Error bars are standard error. HP- Hadley Point and BC-Berry Cove June (n=6), Berry Cove July (n=5).

Tables 3-6 depict the presence/absence of taxa and average abundances observed from the collection of larger organisms and the associated small organisms on the larval collector plates. Tables 3 and 4 correspond with the plates that were in the field from July to August, while Tables 5 and 6 compare plates that were in the field from June to August with those from July to August. The only glaring differences in abundances are at Berry Cove, where mussel seed had a very high abundance in the BC June sample. Again, this could be a reflection of the amount of hydroid that was subsampled and preserved, as a higher number of mussel seed would have been found with a higher amount of hydroid. In addition, the Bar had a low average abundance for mussel seed on the plates, which could also be a reflection of the lack of hydroid.

Table 3. Presence/absence of taxa representing "larger" organisms and the associated small organisms that were observed on larval collector plates that were in the field from July to August. The Bar and BCE-Berry Cove Eelgrass are the only two eelgrass sites. Bar (n=5), BCE-Berry Cove Eelgrass (n=6), HP-Hadley Point sites 1-3 (n=6), BC-Berry Cove (n=5). Presence at each site denoted by x.

Phylum	Class	Family	Taxon identifed	BAR	BCE	HP1	HP2	HP3	BC
			Algae			x	x	x	
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod A	x		x	x	x	x
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod AM				x		
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod B	x			x		
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod C				x		
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod D				x		
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod E				x		
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod F						
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod G	x					
Echinodermata	Asteroidea	Asteriidae	*Asterias* spp	x		x			x
Cnidaria	Hydrozoa	Campanulariidae	Campanulariidae		x	x	x	x	x
Arthropoda	Maxillopoda	(subclass Copepoda)	Copepoda		x	x	x	x	x
Mollusca	Gastropoda	Calyptraeidae	*Crepidula fornicata*	x		x	x	x	x
Mollusca	Gastropoda		Gastropod unid			x			
Annelida	Polychaeta	Polynoidae	*Harmothoe extenuata*			x		x	x
Annelida	Polychaeta	Polynoidae	*Harmothoe imbricata*	x	x	x		x	
Annelida	Polychaeta	Polynoidae	*Lepidonotus squamatus*	x					
Mollusca	Gastropoda	Littorinidae	*Littorina littorea*	x		x		x	x
Mollusca			Mollusca unid					x	
Mollusca	Bivalvia	Mytilidae	*Mytilus edulis* seed	x	x	x	x	x	x
Annelida	Polychaeta	Spionidae	*Polydora* sp					x	
Arthropoda	Maxillopoda	Archaeobalanidae	*Semibalanus balanoides*		x	x		x	x
Arthropoda	Malacostraca	(order Decapoda)	Shrimp unid			x			
Mollusca	Gastropoda	Tergipedidae	*Tergipes tergipes*			x			x
Mollusca	Gastropoda	Lottiidae	*Testudinalia testudinalis*	x				x	
Cnidaria	Hydrozoa	Tubulariidae	Tubulariidae			x			
Platyhelminthes	Turbellaria		Turbellaria						

Table 4. Average abundance per plate of the taxa representing "larger" organisms and the associated small organisms that were observed on larval collector plates that were in the field from July to August. The Bar and BCE-Berry Cove Eelgrass are the only two eelgrass sites. Bar (n=5), BCE-Berry Cove Eelgrass (n=6), HP-Hadley Point sites 1-3 (n=6), BC-Berry Cove (n=5). P= Present.

Phylum	Class	Family	Taxon identifed	BAR	BCE	HP1	HP2	HP3	BC
			Algae			P	P	P	
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod A	0.20		0.67	0.83	1.67	0.20
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod AM				0.17		
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod B	0.40			0.83		
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod C				0.17		
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod D				0.17		
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod E				0.17		
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod F						
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod G	0.60					
Echinodermata	Asteroidea	Asteriidae	*Asterias* spp	1.00		0.17			0.20
Cnidaria	Hydrozoa	Campanulariidae	Campanulariidae		P	P	P	P	P
Arthropoda	Maxillopoda	(subclass Copepoda)	Copepoda		0.33	3.33	6.00	5.83	0.80
Mollusca	Gastropoda	Calyptraeidae	*Crepidula fornicata*	0.20		0.17	0.17	1.00	0.40
Mollusca	Gastropoda		Gastropod unid			0.33			
Annelida	Polychaeta	Polynoidae	*Harmothoe extenuata*			0.50		0.67	0.40
Annelida	Polychaeta	Polynoidae	*Harmothoe imbricata*	0.40	0.17	0.33		0.33	
Annelida	Polychaeta	Polynoidae	*Lepidonotus squamatus*	0.40					
Mollusca	Gastropoda	Littorinidae	*Littorina littorea*	0.20		0.67		1.00	0.20
Mollusca			Mollusca unid					0.17	
Mollusca	Bivalvia	Mytilidae	*Mytilus edulis* seed	0.20	3.50	9.00	6.67	5.67	8.60
Annelida	Polychaeta	Spionidae	*Polydora* sp					0.83	
Arthropoda	Maxillopoda	Archaeobalanidae	*Semibalanus balanoides*		0.17	1.50		0.50	1.20
Arthropoda	Malacostraca	(order Decapoda)	Shrimp unid			0.17			
Mollusca	Gastropoda	Tergipedidae	*Tergipes tergipes*			0.33			0.40
Mollusca	Gastropoda	Lottiidae	*Testudinalia testudinalis*	0.20				0.17	
Cnidaria	Hydrozoa	Tubulariidae	Tubulariidae			P			
Platyhelminthes	Turbellaria		Turbellaria						

Table 5. Presence/absence of taxa representing "larger" organisms and the associated small organisms that were observed on larval collector plates that were in the field either from June to August ("June") or from July to August ("July"). HP-Hadley Point sites 1-3 (n=6), BC-Berry Cove June (n=6) Berry Cove July (n=5). Presence at each site denoted by x.

Phylum	Class	Family	Taxon identified	HP1 JUNE	HP1 JULY	HP2 JUNE	HP2 JULY	HP3 JUNE	HP3 JULY	BC JUNE	BC JULY
			Algae		x	x	x		x	x	
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod A	x	x	x	x	x	x		x
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod AM				x	x			
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod B			x	x	x			
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod C				x				
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod D				x				
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod E				x				
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod F			x					
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod G								
Echinodermata	Asteroidea	Asteriidae	*Asterias* spp	x	x						x
Cnidaria	Hydrozoa	Campanulariidae	Campanulariidae	x	x	x	x	x	x	x	x
Arthropoda	Maxillopoda	(subclass Copepoda)	Copepoda		x	x	x	x	x	x	x
Mollusca	Gastropoda	Calyptraeidae	*Crepidula fornicata*	x	x	x	x	x	x	x	x
Mollusca	Gastropoda		Gastropod unid	x	x	x					
Annelida	Polychaeta	Polynoidae	*Harmothoe extenuata*	x	x	x		x	x	x	x
Annelida	Polychaeta	Polynoidae	*Harmothoe imbricata*		x	x			x	x	
Annelida	Polychaeta	Polynoidae	*Lepidonotus squamatus*								
Mollusca	Gastropoda	Littorinidae	*Littorina littorea*	x	x			x	x		x
Mollusca			Mollusca unid						x		
Mollusca	Bivalvia	Mytilidae	*Mytilus edulis* seed	x	x	x	x	x	x	x	x
Annelida	Polychaeta		Polychaete unid							x	
Annelida	Polychaeta	Spionidae	*Polydora* sp	x		x		x	x	x	
Arthropoda	Maxillopoda	Archaeobalanidae	*Semibalanus balanoides*	x	x	x		x	x	x	x
Arthropoda	Malacostraca	(order Decapoda)	Shrimp unid		x						
Mollusca	Gastropoda	Tergipedidae	*Tergipes tergipes*	x	x					x	x
Mollusca	Gastropoda	Lottiidae	*Testudinalia testudinalis*						x		
Cnidaria	Hydrozoa	Tubulariidae	Tubulariidae		x						
Platyhelminthes	Turbellaria		Turbellaria			x					

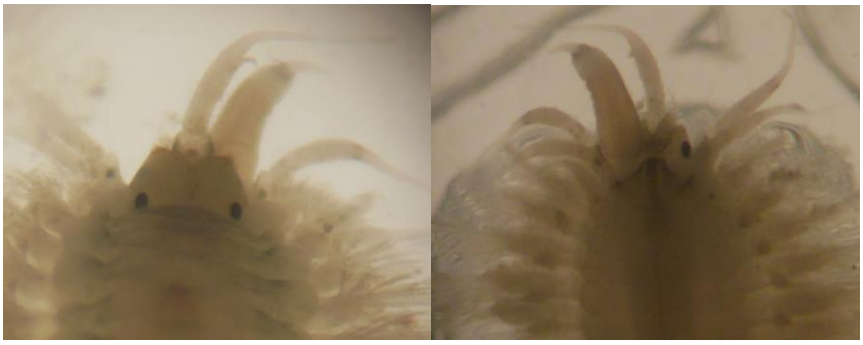
Table 6. Average abundance per plate of the taxa representing "larger" organisms and the associated small organisms that were observed on larval collector plates that were in the field either from June to August ("June") or from July to August ("July"). HP-Hadley Point sites 1-3 (n=6), BC-Berry Cove June (n=6) Berry Cove July (n=5). P = Present.

Phylum	Class	Family	Taxon identified	HP1 June	HP1 July	HP2 June	HP2 July	HP3 June	HP3 July	BC June	BC July
			Algae		P	P	P		P	P	
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod A	0.17	0.67	0.33	0.83	4.33	1.67		0.20
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod AM				0.17	0.67			
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod B			0.83	0.83	0.17			
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod C				0.17				
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod D				0.17				
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod E				0.17				
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod F			0.17					
Arthropoda	Malacostraca	(order Amphipoda)	Amphipod G								
Echinodermata	Asteroidea	Asteriidae	*Asterias* spp	0.17	0.17						0.20
Cnidaria	Hydrozoa	Campanulariidae	Campanulariidae	P	P	P	P	P	P	P	P
Arthropoda	Maxillopoda	(subclass Copepoda)	Copepoda		3.33	3.67	6.00	2.17	5.83	5.00	0.80
Mollusca	Gastropoda	Calyptraeidae	*Crepidula fornicata*	0.33	0.17	0.33	0.17	0.50	1.00	0.17	0.40
Mollusca	Gastropoda		Gastropod unid	0.17	0.33	0.17					
Annelida	Polychaeta	Polynoidae	*Harmothoe extenuata*	0.33	0.50	0.33		0.33	0.67	0.67	0.40
Annelida	Polychaeta	Polynoidae	*Harmothoe imbricata*		0.33	0.17			0.33	0.17	
Annelida	Polychaeta	Polynoidae	*Lepidonotus squamatus*								
Mollusca	Gastropoda	Littorinidae	*Littorina littorea*	1.33	0.67			0.67	1.00		0.20
Mollusca			Mollusca unid						0.17		
Mollusca	Bivalvia	Mytilidae	*Mytilus edulis* seed	6.50	9.00	1.83	6.67	7.33	5.67	36.00	8.60
Annelida	Polychaeta		Polychaete unid							0.17	
Annelida	Polychaeta	Spionidae	*Polydora* sp	0.17		0.33		0.83	0.83	1.00	
Arthropoda	Maxillopoda	Archaeobalanidae	*Semibalanus balanoides*	6.17	1.50	2.83		2.00	0.50	30.67	1.20
Arthropoda	Malacostraca	(order Decapoda)	Shrimp unid		0.17						
Mollusca	Gastropoda	Tergipedidae	*Tergipes tergipes*	0.17	0.33					1.83	0.40
Mollusca	Gastropoda	Lottiidae	*Testudinalia testudinalis*						0.17		
Cnidaria	Hydrozoa	Tubulariidae	Tubulariidae		P						
Platyhelminthes	Turbellaria		Turbellaria			0.17					

Commonly observed organisms

Scale worms observed on collector plates

Lepidonotus squamatus



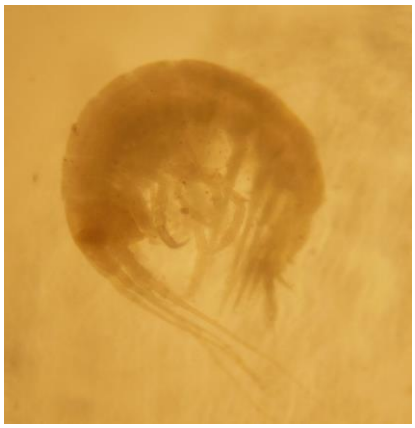
Harmothoe imbricata (dorsal and ventral)

Harmothoe extenuata

Amphipods observed on collector plates

Amphipod A

Amphipod AM – possibly a male of type A

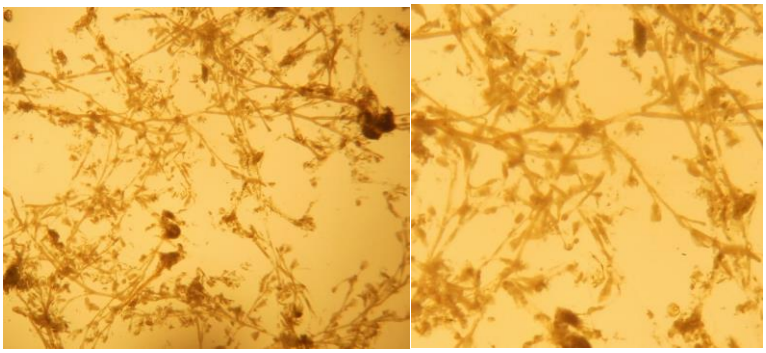


Amphipod B

Amphipod F

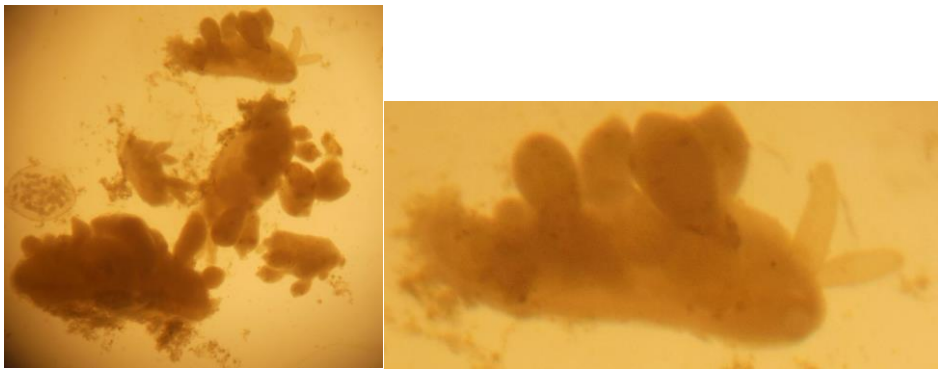
Amphipod G

Other organisms observed on collector plates

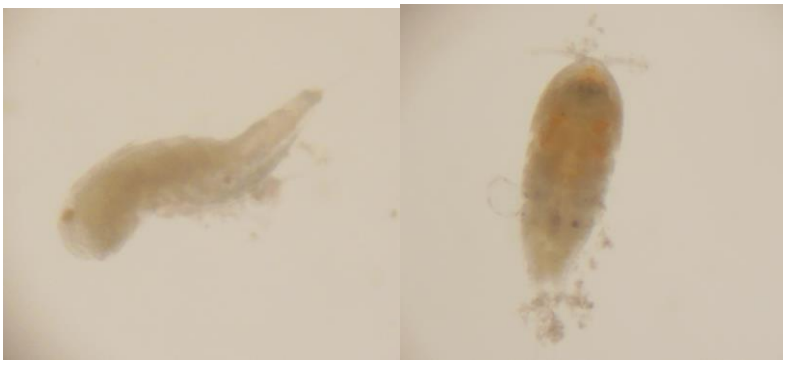


Campanulariidae (e.g. *Obelia bidentata*), right is close up of the same view

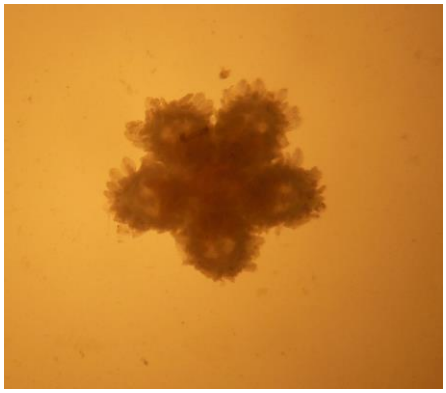
Polydora cornuta (left-specimen from core sample, right-specimen from plate)



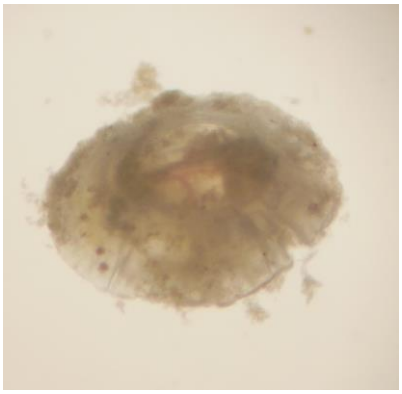
Tergipes tergipes (found associated with campanularian hydroids)



Copepoda

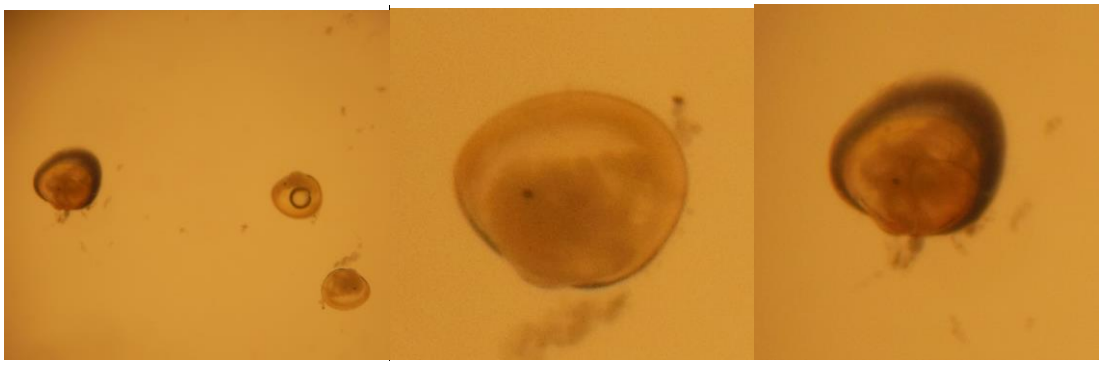


Asterias spp.



Semibalanus balanoides

Crepidula fornicata (shell is chipped)



Mussel seed (individual images to the right are close-ups of the image on the left)

VI. Mussel Coverage

The blue mussel, *Mytilus edulis,* is a species of commercial importance in Frenchman Bay and it occurs within the eelgrass restoration areas. We surveyed percent cover of mussels as a way of documenting this species' occurrence in these areas. Blue mussels can form extensive beds and they add structural complexity to the mudflat habitat. We therefore took note of the fauna associated with this species.

Field Sampling

All sampling was conducted at low tide. At each site, two 60 x 60cm quadrats were thrown randomly at three points along the shoreline and a photograph was taken of each quadrat for later quantification of mussel coverage. From each quadrat, three randomly selected mussels were measured from the umbo to the posterior along the dorsal edge. The larval collector poles were used as a point of reference for sampling. At Hadley Point, sampling was conducted ~10m or more inshore from the array of larval collector poles and quadrats were thrown at three points moving parallel across the shoreline and in line with every other pole in the row of six poles (Figure 9A). At Berry Cove, the collector poles were still in the three rows of four configuration and quadrats were thrown in relation to the first three rows of poles (Figure 9B).

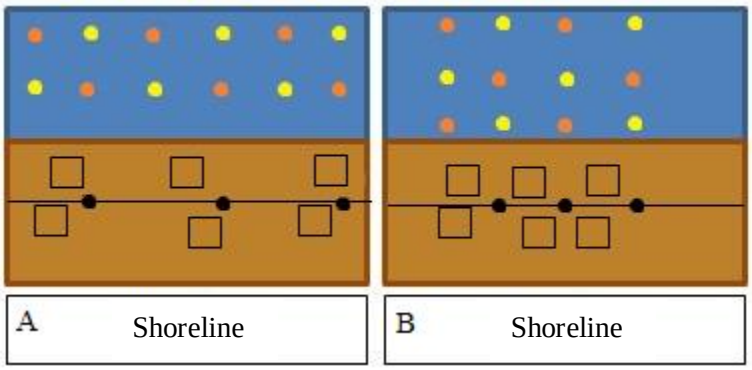


Figure 9. Mussel coverage was documented A) at Hadley Point and B) at Berry Cove by randomly throwing two 60 x 60cm quadrats at three points in line with the larval collector poles (orange and yellow dots). The black dots on the brown background represent the points from which two quadrats (black squares) were thrown randomly on the mudflat. At Hadley Point, surveying was conducted ~10m or more inshore from the collector poles closest to shore.

Sample Processing in the Lab

The photograph of every quadrat was opened in the program ImageJ and a grid was overlain on the image to determine percent cover of mussels within the quadrat (Figure 10). Percent cover was determined by counting the number of squares occupied by mussels (counts were not rounded to whole squares) and dividing by the number of squares encompassing the area within the quadrat. Other organisms and features that occurred within the quadrat were also recorded.

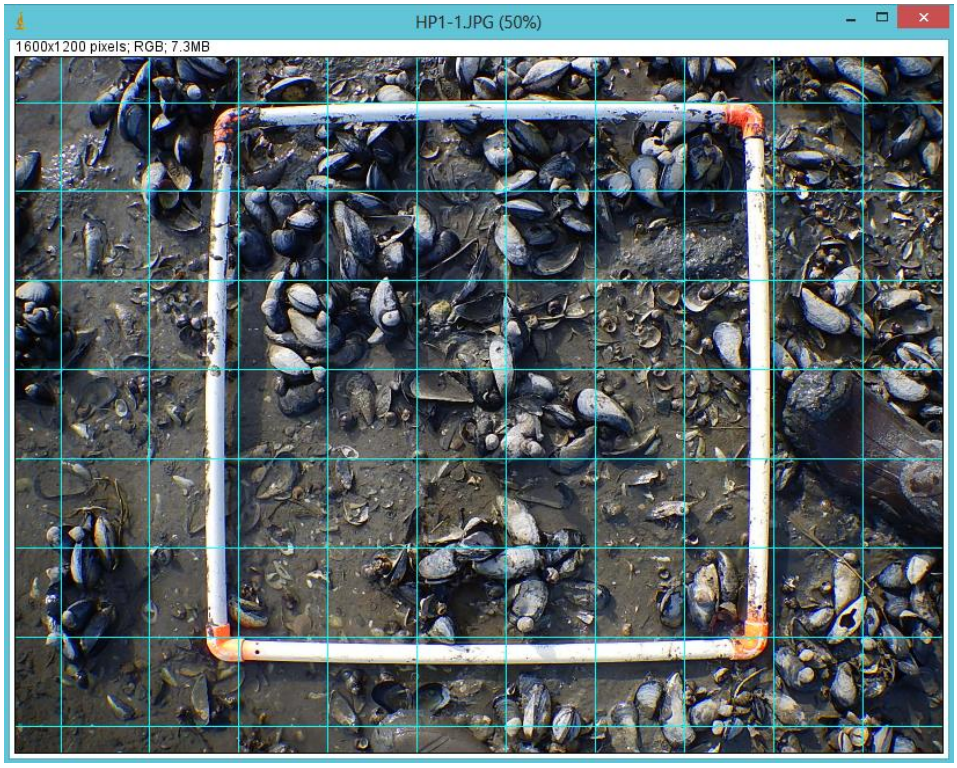


Figure 10. Percent cover of mussels was determined by overlaying a grid on each quadrat photo in the program ImageJ.

Data Analysis

Data were entered into a Microsoft Access database and exported to Microsoft Excel. The average percent mussel cover for each of the four sites was determined and percent cover was compared among sites using the non-parametric Kruskal-Wallis test, as the data did not fit ANOVA's assumptions of normality and homogeneity of variance. Analyses were conducted using R and Microsoft Excel.

Results

Kruskal-Wallis revealed that the differences in mussel percent cover among sites was not significant (χ^2= 5.56, df = 3, p= 0.135), though it is clear that the quadrats thrown at Berry Cove had consistently lower mussel coverage (ranging from 0-6%), while Hadley Point 3 had a very large range in coverage (from 0-83%) (Figure 11). As we sampled only a small area with six quadrats, we didn't capture the mussel coverage for the whole area. Mussel coverage could be characterized more accurately in future surveys by throwing a greater number of quadrats over a much broader area within each of the four restoration sites. Average mussel size from the four sites ranged from 9.7-11.3 mm (Figure 12). The fauna, flora, and features that were observed within the quadrats assessed for mussel coverage are presented in Table 7. Positive identification and verification that organisms (particularly shelled organisms) were alive was limited by what was discernible in the photograph.

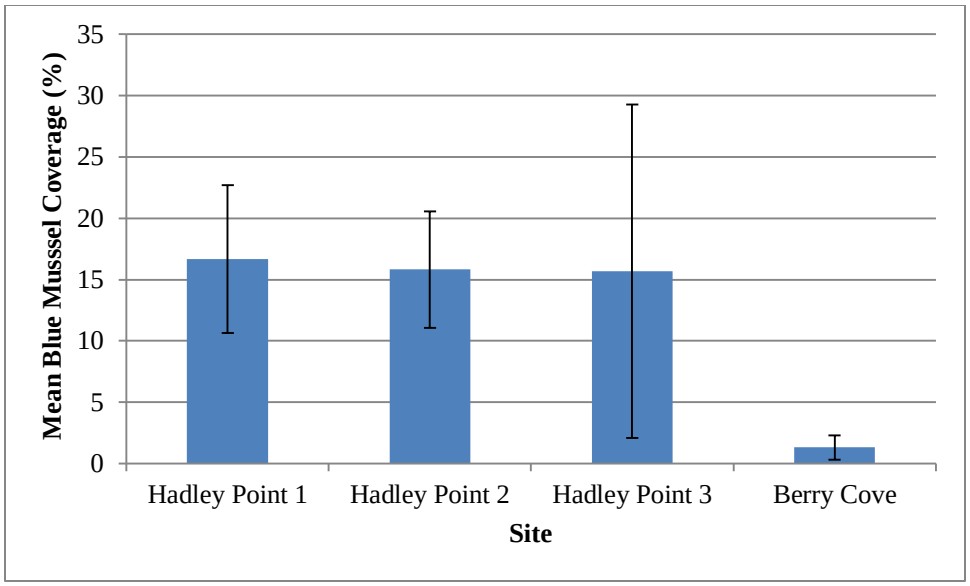


Figure 11. Average mussel coverage per 60 x 60cm quadrat (n=6). Error bars are standard error.

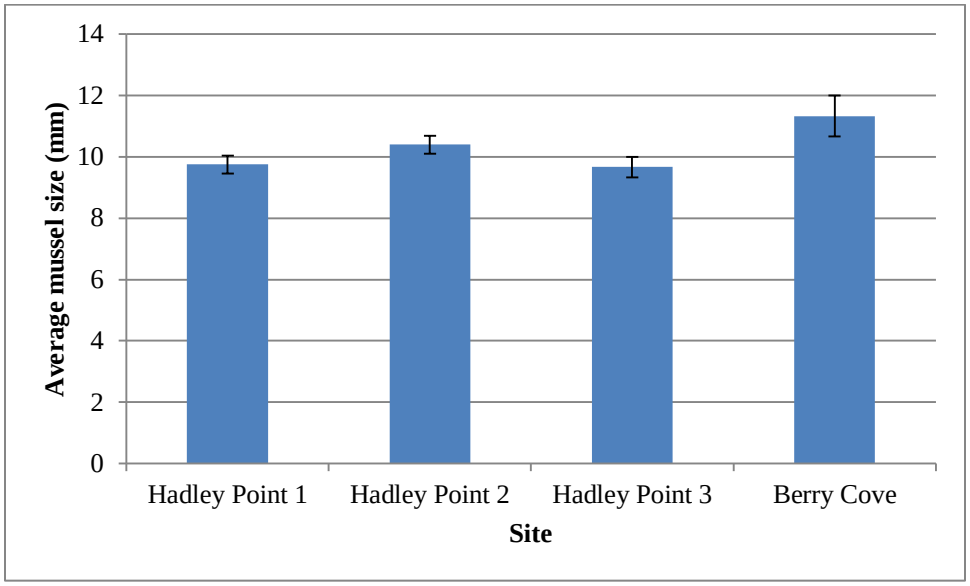


Figure 12. Average mussel size per site based on quadrats where mussels were present. Hadley Point 1 (n=4), Hadley Point 2 (n=5), Hadley Point 3 (n=2), Berry Cove (n=2). Mussels were measured from the umbo to the posterior along the dorsal edge.

Table 7. Additional fauna, flora, and features observed within the quadrats thrown to document blue mussel coverage in the eelgrass restoration areas. Fauna and flora were observed attached to rocks and/or shells (including mussel shells) or were observed directly on the mud's surface.

Additional fauna and flora observed	Features observed
Periwinkle, *Littorina littorea*	Bird footprints
Slipper limpet, *Crepidula fornicata*	Rocks
Barnacle, likely *Semibalanus balanoides*	Shells
Knotted wrack, *Ascophyllum nodosum*	Clam burrows
Possibly the tortoise-shell limpet, *Testudinalia testudinalis*	Small burrows (possibly clam?)
Whelk (possibly just the shell), likely *Buccinum undatum*; and possibly a second species present (unidentified)	Drift wood
Starfish, *Asterias* spp.	Oak leaf
Sand shrimp, *Crangon septemspinosa*	Sea foam
Possible razor clam, *Ensis directus*, or just a crab claw	
Straw-like plant material (possibly terrestrial grass)	

VII. Seining

Seining was used as a method to sample mobile organisms living in the water column and above the sediment surface.

Field Sampling

The seine net that was used for sampling was stretched between two wooden poles and measured ~3.45m wide and 1.2m tall with a 0.25in mesh. At each site, six sweeps were conducted 10m toward shore starting in ~90-110cm of water. Three sweeps were conducted on either side of the larval collector pole arrays (Figure 13). We tried to start in line with one of the deepest two rows of poles (in relation to the original configuration of three rows of four). Sweeps were conducted 10m apart unless there was some kind of obstruction that required moving a greater distance apart between

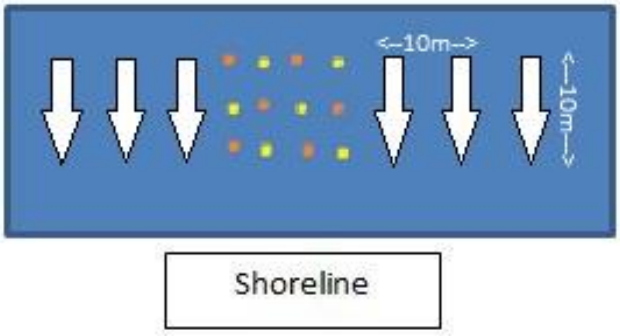


Figure 13. Seining sampling design in eelgrass restoration areas. Three sweeps were conducted on either side of the larval collector pole array (orange and yellow dots). Arrows represent direction and location of sweep.

sweeps. The 10m distance was marked from the starting point by one person planting a PVC pole with a 10m long string in the sediment. This person walked with the string along with the two people conducting the seine sweep. We used a different string to connect the tops of the two seine net poles so that by keeping this string pulled taut during the sweep, we ensured that each sweep covered the same distance across with the net. In addition, the seiners needed to make sure that the net stayed on the bottom as it was pulled along and that the bottom line of the net never fell behind the top of the net with the buoys. When 10m toward the shore was reached, the person walking with the 10m marker string indicated to the seiners to stop. The seiners then scooped the net forward toward shore in a semi-circular motion until the poles were held parallel to the surface of the water. The net was then walked into shore and laid on the ground and organisms were quantified and recorded (Figure 14). Fish and shrimp were held in containers with seawater as they were picked up and counted. Species that could not immediately be identified were either photographed or brought back to the lab in seawater.

Sample processing and data analysis

Any organisms that were not identified in the field were identified using a taxonomic guide (Pollock, 1998). Data were entered into a Microsoft Access database and exported to Microsoft Excel. Analyses and graphical and tabular presentation of the data were conducted in Microsoft Excel.

Figure 14. Conducting a seine sweep. A) Walk 10m slowly toward shore B) scoop seine net into a horizontal position C) walk net and contents to shore D) sort, identify, and quantify organisms and return them to sea. Liz Thompson, Lukas Thorburn, and Shannon White pictured here.

Results

A total of eight taxa were collected during the seining surveys at Berry Cove and Hadley Point (Table 8). Green crabs and the sand shrimp, *Crangon septemspinosa,* were caught at all of the sites. *Crangon septemspinosa* was caught in the highest abundance across sites, followed by periwinkles, and then the green crab. Juvenile flounder, slipper limpets, and one Atlantic silverside were collected only at Berry Cove, while sticklebacks were only collected at Hadley Point. Organism abundance was particularly high for our very first seining survey at Berry Cove. This survey took place in late June and the weather was windy and the water was turbid and wavy on that day. The wind and waves may have churned up the water and brought a lot of organisms off the bottom and into the water column. In addition, the organisms wouldn't have been able to see us coming easily and were probably less able to avoid the net than in the relatively calmer and clearer conditions we saw during the remainder of our seining surveys. With the exception of Berry Cove June, the mean number of taxa collected fell between 1 and 3 for the remaining sites (Figure 15). By far, the highest number of individuals was captured at Berry Cove in June (Figure 16). With the exclusion of this survey, Berry Cove still had the highest average for the total number of individuals with 74 individuals caught overall, while at Hadley Point Area 2 only 10 individuals total were caught. The difference in the number of organisms collected may be a reflection of the spatial variation among the Berry Cove and Hadley Point sites, or perhaps a higher number of seine sweeps would reveal an evening out of the numbers among sites.

Table 8. Average abundance per seine sweep of taxa captured during seining surveys at Berry Cove (BC) and Hadley Point (HP). Berry Cove was sampled twice due to inclement sampling conditions on the first attempt to conduct six sweeps at the end of June (BC June). Only three sweeps were made (n=3) at Berry Cove in June. The remainder of the seining was conducted in early July with n=6 sweeps at each site.

Phylum	Class	Family	Scientific name	Taxon identified	BC June	BC	HP1	HP2	HP3	Notes
Arthropoda	Malacostraca	Portunidae	*Carcinus maenas*	Green Crab	6.33	0.67	0.17	0.50	1.00	
Arthropoda	Malacostraca	Crangonidae	*Crangon septemspinosa*	Sand shrimp	153.33	8.83	5.00	1.17	3.67	
Mollusca	Gastropoda	Calyptraeidae	*Crepidula fornicata*	Slipper limpet	0.67					Slipper limpets were on a rock.
Chordata	Actinopterygii	Gasterosteidae	*Gasterosteus aculeatus* or *G. wheatlandi*	Stickleback			0.17		0.17	Tentatively identified as three-spine stickleback (*G. aculeatus*) although it could be a two-spine stickleback (*G. wheatlandi).*
Mollusca	Gastropoda	Littorinidae	*Littorina littorea*	Periwinkle	6.00	2.17	0.67		0.33	
Chordata	Actinopterygii	Atherinopsidae	*Menidia menidia*	Silverside	0.33					
Arthropoda	Malacostraca	Mysidae	*Mysis* sp	Mysid shrimp		0.33			0.17	
Chordata	Actinopterygii	Pleuronectidae	*Pseudopleuronectes americanus*	Juvenile flounder	3.33	0.33				Most likely winter flounder (*P. americanus*).

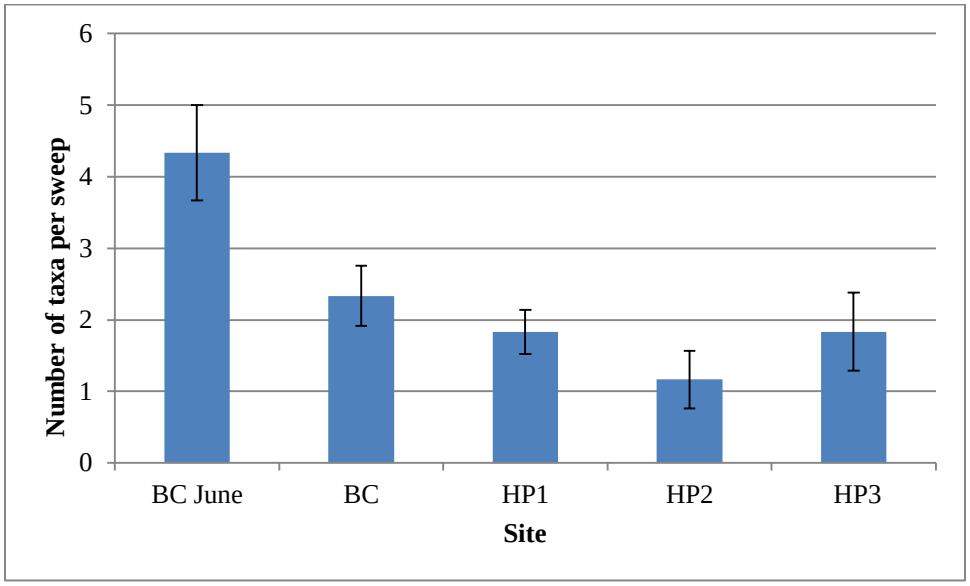


Figure 15. Average number of taxa per seine sweep by site. Only three sweeps were made (n=3) at Berry Cove in June. The remainder of the seining was conducted at Berry Cove (BC) and Hadley Point (HP)in early July with n=6 sweeps at each site. Error bars are standard error.

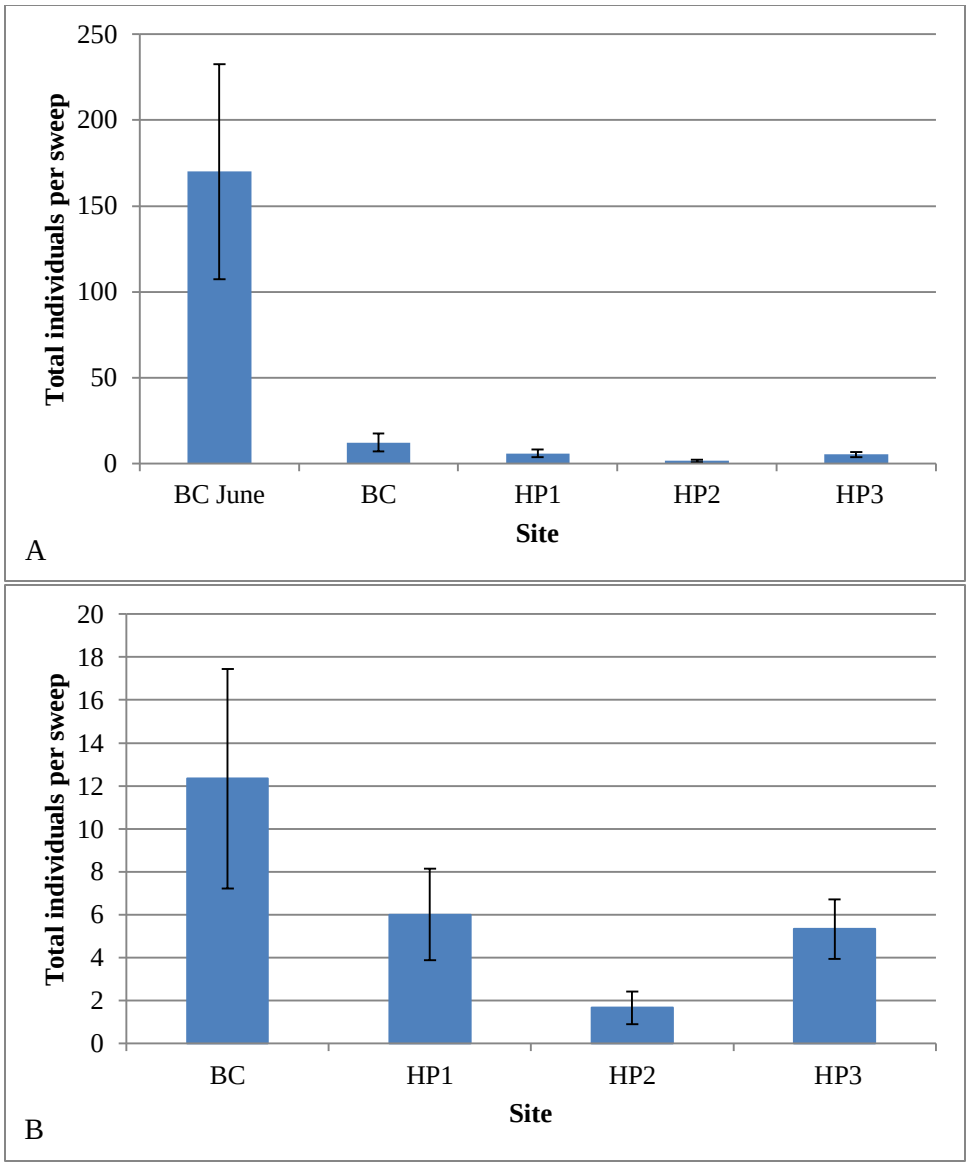


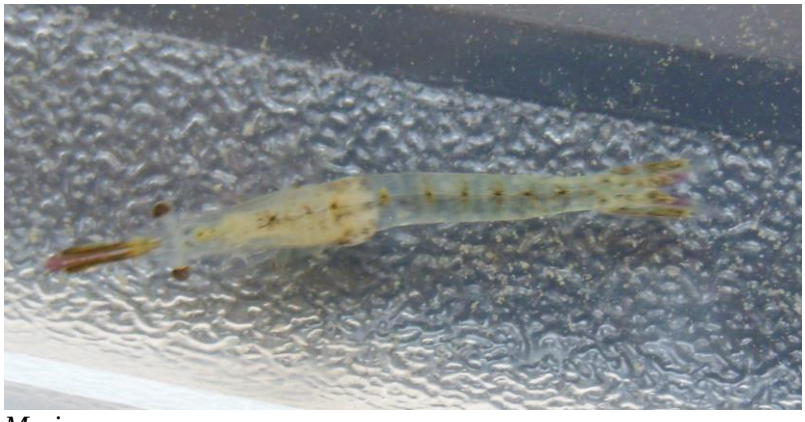
Figure 16. Average total number of individuals per seine sweep by site. Only three sweeps were made (n=3) at Berry Cove in June. The remainder of the seining was conducted at Berry Cove (BC) and Hadley Point (HP) in early July with n=6 sweeps at each site. A) BC June included, B) BC June excluded. Error bars are standard error.

Organism images from seining

Crangon septemspinosa

Stickleback

Green crab, *Carcinus maenas*



Mysis sp.

VIII. Sampling timeline

This timeline covers sample collection or equipment deployment for the Habitat Function Project only and does not include dates of field equipment trials or other field reconnaissance necessary for this or other CEHL projects.

6/11/13-Larval collectors deployed in three rows of four at Berry Cove and at Hadley Point Areas 1-3

6/24/13-Berry Cove infaunal core sampling

6/25/13-Hadley Point infaunal core sampling

6/27/13-Berry Cove Seining (first try-inclement sampling conditions)

7/2/13-Seining at Berry Cove and Hadley Point Area 1

7/3/13-Seining at Hadley Point Areas 2 and 3

7/9/13-Hadley Point larval collector plate replacement and redeployment (two rows of six set seaward of original array)

7/10/13-Berry Cove larval collector plate replacement and deployment (poles returned to original location)

7/10/13-Berry Cove Eelgrass larval collectors deployed (two in each of three patches) and infaunal core sampling carried out

7/12/13-The Bar larval collectors redeployed with new plates in eelgrass (originally deployed by Ted)

7/25/13-Hadley Point mussel coverage survey

7/26/13-Berry Cove mussel coverage survey

8/7/13-The Bar larval collectors in eelgrass retrieved

8/8/13- Retrieval of larval collectors at Berry Cove, Berry Cove Eelgrass, and Hadley Point

IX. References

Pollock, LW. 1998. A Practical Guide to the Marine Animals of Northeastern North America. Rutgers University Press, New Brunswick, 367 pp.