Restored Living Shorelines

A comparison of ecosystem services relative to natural marshes.

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Restored Living Shorelines: a comparison of ecosystem services relative to natural marshes.

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Website on this project and related work:
https://vaudrey.lab.uconn.edu/living-shorelines/

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Matching funds were provided by the DuPont Corporate Remediation Group. DuPont contributed in-kind services for site security and facilities maintenance at the site. Their site ecological restoration efforts are voluntary activities that are not required by regulatory mandates. DuPont did not otherwise contribute to our efforts nor review this report.

Any opinions, findings, conclusions, or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the sponsors.

The authors have chosen to use the original name of *Spartina alterniflora* Loisel. versus the newer taxonomic name, *Sporobolus alterniflorus* (Loisel.) P.M. Peterson & Saarela, identified in 2014; changing to this new name are contested (Bortolus et al. 2019).

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1 Executive Summary

Several initiatives have advocated for more studies of the blue carbon[1] potential of marshes and other wetland habitats, and attempted to determine a path towards commercializing carbon sequestration and storage as a commodity (Howard et al. 2017; Rodosta et al. 2011). Within the last five years, the Intergovernmental Panel on Climate Change (IPCCC) has developed guidelines for assessing coastal blue carbon stores and have included them in their carbon accounting (IPCC 2014). As society becomes more aware of the importance of reducing our collective carbon footprint, accurately assessing and managing blue carbon stores is going to become increasingly important. Restored marshes have the potential to contribute to carbon storage, but in urbanized estuaries like Long Island Sound where space is limited and many of our marshes are previously disturbed, the question becomes, "do these marshes deliver the same benefits and how long does it take a restored marsh to achieve parity with natural, mature marshes?"

A recently established living shoreline in Stratford, CT, USA provides a unique opportunity to compare the ecosystem services of two newly planted fringing saltmarshes (2014, 2017) to nearby established fringing and meadow marshes in the same estuary. By definition, a fringing marsh occurs along estuarine shorelines, are relatively narrow with a gentle grade from open water to upland, have less area of high marsh, and are more exposed to wind and wave energy than other types of marsh (Cook et al. 1993). In contrast, meadow marshes develop in low energy areas, are relatively large, contain more than 50% high marsh, and develop a distinct bank between open water and the marsh (Cook et al. 1993).

Figure 1: Stratford Point Living Shoreline Restoration
Reef balls are arrayed in a zig zag pattern to the left of the photo. Multiple lines have been established and are visible in the distance (Figure 7). Spartina alterniflora *planted four years ago is established landward of the reef balls. In the distance to the right, dead trees have been fixed to the beach to improve sediment retention and a dune system has been established at the transition to the upland area.*

The Stratford Point living shoreline restoration is a collaborative effort between the corporate landowner (Dow-DuPont), non-profit organizations (National Audubon Connecticut, Connecticut Audubon Society, The Nature Conservancy), local town officials (Stratford) and Sacred Heart University. Sacred Heart University oversees the restoration efforts, monitoring of the site, and runs educational

[1] Blue carbon is carbon fixed by coastal ocean ecosystems, rather than terrestrial systems.

programs and outreach events. Daily oversight and access to the site was provided by Connecticut Audubon Society and currently by Audubon Connecticut. Funds for the initial restoration (start date: April 2014) were provided by National Fish and Wildlife Foundation (NFWF) and DuPont Corporation. The expansion of the restoration (start date: November 2016) was funded by DuPont, NFWF, and CIRCA. The continuing restoration project was funded by the CT In-Lieu Fee Fund, DuPont, NFWF, and NOAA and currently includes over 305 m of artificial oyster reef (reef balls), newly planted low marsh smooth cordgrass (*Spartina alterniflora* Loisel.), high marsh saltmeadow cord grass (*Spartina patens* (Aiton) Muhl.), contoured dune system and upland coastal meadows, shrub, and forest mosaic (Figure 5).

The reference fringing and meadow marshes located in the same estuary were compared to the restored fringing marsh to assess differences in carbon sequestration. Biodiversity indices at the three sites were also compared. We hypothesized that within five years, ecosystem services of the restored fringing marshes would be similar to naturally occurring fringing marshes but less than the meadow marsh. The work conducted under this grant establishes a baseline, as both marshes are less than five years old. We further predicted that seasonal variation in carbon content of the restored marsh would be greater than that of the reference marshes, due to the more dynamic sedimentary environment of the restored marsh, as it matures.

Stations were established at six sites in the mouth of the Housatonic River, CT (Figure 2). The restoration at Stratford Point includes an older section of reef balls and plantings established in 2014 (ID: Stratford Point North) and a newer section of reef balls and plantings established in 2017 (ID: Stratford Point South).

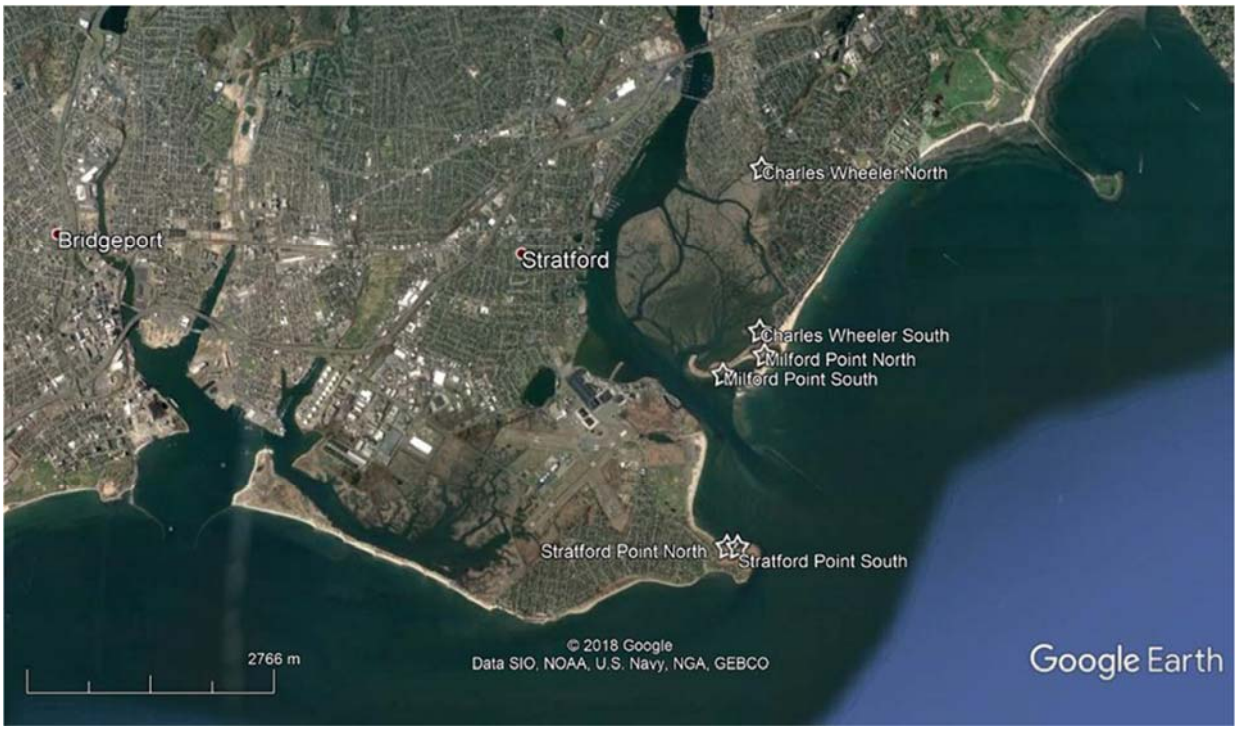

Figure 2: Overview of Sampling Site Locations
Sampling sites are designated by white stars. Stratford Point North and South are the restored fringing marshes. Charles Wheeler North is the meadow marsh. The remaining marshes are established fringing marshes. Four to six stations were sampled per site (Table 2, Figures 7 to 11).

Carbon sequestration was measured as a function of sediment accumulation rate and sediment carbon density, which is calculated from soil bulk density (g cm^{-3}) and percent carbon in soil (Chmura et al. 2003; Craft et al. 1991). All stations were sampled in June of 2018 (within three days of 6/20/18) to assess the average and spatial variability within each site. Two stations within each site were sampled on four dates between April and October of 2018 to assess the temporal variability – to see if the date of sampling impacts our estimate of carbon sequestration rate.

Fish utilization of the natural fringing marsh at Milford Point North and Milford Point South were compared to Stratford Point by trapping fish in baited minnow traps. Video cameras were deployed at Milford Point South and Stratford Point to get a better understanding of fish utilization of the reef over time, using a non-invasive technique (Figure 3). In the future, fish sampling protocols should be revised to better compare the Stratford Point marsh to the Milford Point marsh, placing all cameras in marsh grass versus sampling the reef balls at Stratford Point and comparing them to the marsh grass at Milford Point. In this pilot study, comparing the reef balls to marsh grass was desirable, to see if the reef balls served a similar function in terms of structure and refuge for fish, compared to marsh grass. In general, the diversity and species abundance was lower at Stratford Point, but this reflects the difference between reef balls and marsh grasses, not the difference between the marshes at the two sites. The video sampling was successful at capturing the community composition and abundance of species and shows great promise as a metric for comparing fish use of these areas.

Figure 3: Image from GoPro Camera 3, Deployed at Milford Point South, 9/18/19
The camera is on the bottom, pointing towards the surface. Note the large school of juvenile bluefish. Two striped killifish are captured in this image, one in the foreground on the right and one under the diagonal stem on left.

The carbon sequestration varies among the sites sampled, with the established natural fringing marshes having average carbon sequestration rates ranging from 88 ± 18 g m^{-2} y^{-1} and 180 ± 5 g m^{-2} y^{-1} (average and standard error). The marsh meadow platform at Charles Wheeler North fell within the range seen at the established fringing marshes, with an average carbon sequestration rate of 112 ± 13 g m^{-2} y^{-1} (Figure 4). The restored marshes (4-year old SN and 1-year old SS) showed lower carbon sequestration rates, with the older restored marsh having a greater sequestration rate than the younger (27 ± 6 g m^{-2} y^{-1} vs. 4 ± 1 g m^{-2} y^{-1}). As these restored marshes age, plant biomass and coverage will increase and should further help to accumulate sediment and carbon in these sites.

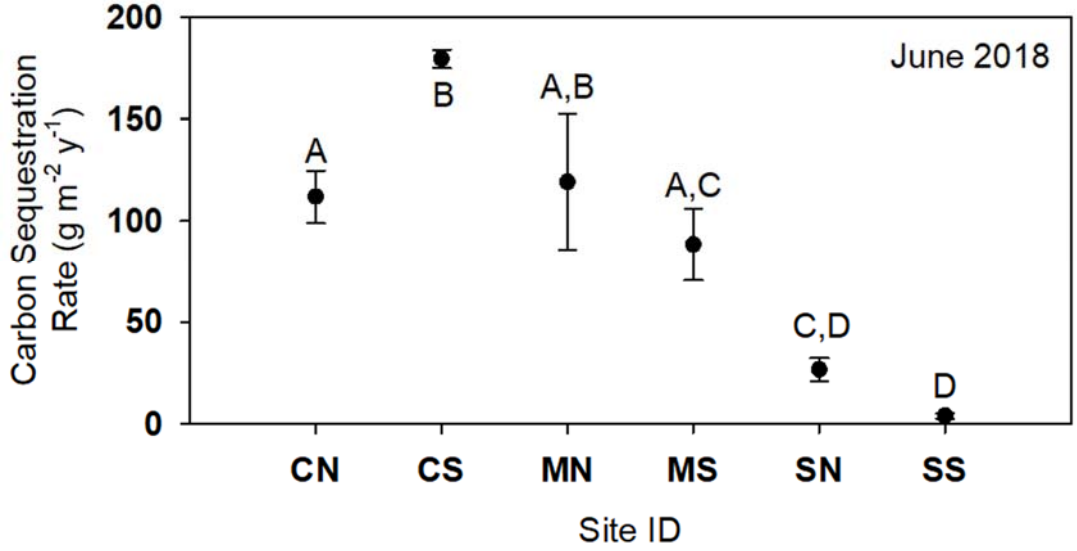

Figure 4: Comparison of June Carbon Sequestration Rates Among Sites
Points represent the average carbon sequestration rates in each site. The number of stations included in each average were: CN = 6, CS = 4, MN = 4, MS = 5, SN = 3, SS = 5. The error bars are the standard error. Letters indicate statistical similarity based on a one-way ANOVA and Holm-Sidak multiple comparisons procedure ($F(5,21) = 14.535$, $p < 0.001$).

The restored fringing marshes at Stratford Point are on their way towards achieving similar levels of carbon sequestration as natural fringing marshes, but as expected by their age (four-years old & one-year old), are still falling short. Recovery time for marshes ranges between five and twenty-five years (Borja et al. 2010; Craft et al. 2003), thus these newly restored marshes are just beginning the journey towards maturity. The dynamic nature of the sediment at the Stratford Point site, a result of wind and wave action, may make this site slower to achieve full parity with natural fringing marshes. The Milford Point South site provides a good reference for Stratford Point as it has a similar rocky soil type and exposure to wind and wave action.

The marsh grasses are expanding through the intertidal area of Stratford Point, suggesting that the restoration efforts are positively impacting the return of a living shoreline to the formerly heavily impacted Stratford Point coastal area. Continued monitoring of Stratford Point will chart its' progress towards developing into a mature and fully functional fringing marsh, providing insight into the trajectory of recovery for living shorelines.

2 Introduction

Sixty years ago, Charles Keeling began taking daily measurements of atmospheric carbon dioxide (CO_2) in an effort to monitor trends in CO_2 levels in the atmosphere. This long term data set established that CO_2 levels in the atmosphere have rapidly been on the rise, increasing from an annual average of 315 ppm (microliters of CO_2 per liter of air) in 1958 to 351 ppm in 1988 (Keeling et al. 1976; Post et al. 1990), and surpassing 400 ppm in 2016 with continuing annual increases predicted (Le Quéré et al. 2018). In comparison CO_2 levels over the past 160,000 years vary between 200 to 300 ppm, and during the years 1750 to 1800 were ~279 ppm (Post et al. 1990). This drastic rise in CO_2 is attributed to anthropogenic effects, with burning of fossil fuels the prime culprit, and has led to decades of work attempting to balance the global carbon budget (Post et al. 1990). Imbalances in the carbon budget due to anthropogenic activities (burning fossil fuels, land use changes, pollution, etc.) have led to more CO_2 in the atmosphere than natural sinks have absorbed (Le Quéré et al. 2018; Post et al. 1990). While reports vary in their estimates of carbon storage potential, most agree that release of carbon to the atmosphere is outpacing storage in natural sinks and there is little hope for current storage capacity of natural sinks to mitigate these carbon increases on their own (Connor et al. 2001; Falkowski et al. 2000).

Coastal habitats are often underrepresented within the global carbon cycle. They have only been considered within the last 20 years (Gattuso et al. 1998), and only within the last 5 years have been included in the global carbon inventory with specific guidelines to assess their potential to store carbon, referred to as blue carbon (IPCC 2014). The most recent IPCC report stresses the need to limit global warming to 1.5°C, and suggests examining pathways to reduce CO_2 in the atmosphere through methods such as carbon capture and storage, afforestation, and reforestation. However, the report does not discuss managing coastal habitats for blue carbon (IPCC 2018).

Tidal wetlands provide a wealth of ecosystem services, including erosion control, storm protection, sequestration of carbon and other nutrients and habitat vital to various life stages of commercially and ecologically important fish and wildlife (Gedan et al. 2009). Coastal habitats have the unique ability to transfer carbon to long term carbon cycles where it is stored for thousands of years (Chmura et al. 2003; McLeod et al. 2011); decomposition of plant material is slowed due to salt water inundation, thus material builds up in sediments, trapping carbon instead of releasing it back into the atmosphere (Chmura et al. 2003; Macreadie et al. 2017). Of coastal habitats, mangroves are the most globally significant for carbon sequestration because of the rate at which they sequester carbon and their total global area (Barbier et al. 2011), however salt marshes are able to sequester carbon at rates disproportionate to their biomass, making them an important player in the global carbon cycle. Marshes store carbon above and below ground in plant biomass, sediments, and peat (Chmura 2013; Radabaugh et al. 2018; Villa and Bernal 2018), with the below-ground storage accounting for 93.5% of the storage capacity (Radabaugh et al. 2018). It is estimated that salt marshes in the contiguous USA account for 31% of the carbon sink of all USA ecosystems (Bridgham et al. 2006).

Tidal marshes are recognized for their unique ability to bury carbon (shift it to the long-term carbon cycle) and have the reported ability to sequester carbon at the same rate or faster than terrestrial forest carbon sinks (Brevik and Homburg 2004; Chmura et al. 2003; McLeod et al. 2011), and 20 times faster than carbon deep sea burial rates (Hopkinson et al. 2012). Estimates of carbon sequestration in marsh ecosystems are highly variable (29 – 210 gC m^{-2} yr^{-1}) due to differences in methodology (Hopkinson et al.

2012) as well as variation between sites related to sediment type, marsh grass species and density, and primary productivity (Chmura et al. 2003). This important ecosystem service has an estimated economic value[2] of \$44.70 ha^{-1} yr^{-1} (Barbier et al. 2011) and restoration of these habitats is being explored as a source of carbon credits (Howard et al. 2017). Given the amount of ecosystem services provided by tidal marshes, restoration of these habitats provides an avenue for mitigating climate change and improving coastal habitats overall.

Thirty years ago restoration ecology was an emerging "crisis discipline" and researchers were working to define the goals and criteria of restoration in various habitats and to identify critical habitats in need of remediation (Cairns Jr and Heckman 1996). Currently, restoration efforts are assessed on their ability to bring a habitat back to its historic state, which sometimes happens, but some ecosystems never reach their pre-disturbance level of function. Carbon stocks are one metric of ecosystem function for tidal marshes. While sediment carbon levels are lower in constructed marshes, younger marshes are able to trap sediment and sequester carbon at rates similar to or faster than natural older marshes when they are first created, although some studies show that these values then plateau (Craft et al. 2003; Zedler and Callaway 1999).

A review of coastal restoration efforts revealed that the full recovery of coastal marine ecosystems can take between 15 and 25 years depending on the type and duration of the disturbance (Borja et al. 2010); this time frame also applies to tidal marshes, as evidenced by a number of examples: Within 5 to 15 years of construction, restored marshes in California, USA have been shown to develop similar levels of ecosystem function as naturally occurring marshes except for soil organic carbon and nitrogen pools (Craft et al. 2003). A study of New England (USA) salt marshes found that there was no difference in reference vs. restored marshes when comparing accretion, organic matter, or inorganic matter, and that restored marshes more quickly accreted sediment compared to reference marshes over a 30-year period (Anisfeld et al. 1999). A study in Connecticut, USA salt marshes showed that within 15 to 20 years restored marshes are functioning similarly to natural ones and had similar levels of biodiversity for plant species, vertebrates, and macroinvertebrates (Warren et al. 2002). Modeling efforts predict restored marshes should function similarly to natural marshes within 5 to 15 years of establishment, including sequestering carbon at similar or faster rates (Craft et al. 2003).

Other studies conducted in North Carolina suggest restored marshes do not provide ecosystem services at comparable levels to natural systems (Zedler 2003). In another comparison, a 10-year old restored marsh in San Diego, CA exhibited 75% less carbon sequestration compared to the nearby naturally occurring marsh (Zedler and Callaway 1999). More studies are needed to fully resolve whether restored marshes provide ecosystem services in levels similar to natural marshes, and it is likely that some restored marshes will match their natural counterparts sooner than others. Restoration may not be beneficial or practical in all areas, for example in Louisiana, USA, restoration efforts are unlikely to reverse the extreme levels of marsh erosion (DeLaune and White 2012). It is important to critically assess habitats before initiating a restoration project, however since restored marshes are shown to sequester carbon, they can play an important role in increasing global blue carbon.

[2] All dollar values are adjusted to US$ 2018 using the US Bureau of Labor Statistics CPI Inflation calculator.

Figure 5: Stratford Point Living Shoreline Restoration
Reef balls are arrayed in a zig zag pattern to the left of the photo. Multiple lines have been established and are visible in the distance (Figure 7). Spartina alterniflora *planted four years ago is established landward of the reef balls. In the distance to the right, dead trees have been fixed to the beach to improve sediment retention and a dune system has been established at the transition to the upland area.*

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Table 1: History of the Restoration Site
Timeline of the history of the restoration efforts at Stratford Point. Stratford Point is owned by Sporting Goods Properties, Inc., a wholly-owned subsidiary of the DuPont Company. The site is protected by a conservation easement held by the State of Connecticut and managed by the Connecticut Audubon Society. For much of the 20th century, it was the site of the Remington Gun Club.

Date	Whole Site at Stratford Point	
	Original Restoration	Expansion
2000-2001	DuPont completed a large remediation project to remove lead shot that had been left over the decades of use as a gun club.	
2001, post-remediation	Planted several thousand *Spartina alterniflora* plugs that were subsequently washed away due to lack of the peat matrix of the marsh, along with the replaced fine sediments. Surviving plant material was denuded by flocks of Canada goose (*Branta canadensis*).	
summer, 2002	Re-seeding of upland area post lead-remediation. Used a mixture of prairie grasses deemed tolerant of local conditions, including native and non-native herbaceous plants.	
December, 2011	Manmade dune established along the north cove, 900 linear feet of six plastic geo-tubes filled with sand, tied, stacked and staked in. Tubes were covered with a sand/20% organic mix. Planted with native dune grass plugs. Hurricane Irene took out the sand and grass before growth occurred (Feb. 2012). More sediment was added and grasses were replanted. Growth occurred for six months and was treated with herbicides to control broadleaf invasives. Hurricane Sandy (Oct. 2012) washed away the sediments and plants. Uncovered geo-tubes break down when exposed to UV radiation.	
February, 2012	Controlled burn to control invasives in upland area, 20 acres.	
May, 2012	Coastal upland restoration, 2 acres. Ninety-six native trees and shrubs planted in four habitat islands (groups). Each grouping consisted of two Northern Hackberry (*Celtis occidentalis,*), two Eastern Red Cedar (*Juniperus virginiana),* four Beach Plum (*Prunus maritime),* four Shadbush *(Amelanchier canadensis),* four Northern Bayberry, *(Myrica pensylvanica),* four Staghorn Sumac *(Rhus typhina),* and five Red Bearberry *(Arctostaphylos uva-ursi).*	
spring, 2014 to 2015	Two sections of rip-rap (150 feet) added to the ends of the geo-tubes. Under the rip-rap, geo-plastic matting was trenched in and buried with gravel. Sand was used to bury the geo-tubes and it subsequently washed away.	
May 6-7, 2014	Sixty-four Pallet Reef Balls™ are installed along a 49-meter stretch. Pallet Reef Balls™ are 4 feet in diameter at the base, 3 feet tall, and weigh 1,500 to 2,000 pounds.	
May – June, 2014	*Spartina alterniflora* salvaged from the Town of Fairfield planted, 130 m^2.	

Date	Whole Site at Stratford Point	
	Original Restoration	Expansion
June, 2015	3,000 *Spartina alterniflora* plugs from Pineland Nursery, NJ planted, 900 m^2.	
November, 2016		274 reef balls (128 Pallet Reef Balls™ and 145 Ultra Reef Balls™) were installed along a 300-meter length of beach. Pallet Reef Balls™ are 4 feet in diameter at the base, 3 feet tall, and weigh 1,500 to 2,000 pounds. Ultra Reef Balls™ are 5.5 feet in diameter at the base, 4.3 feet tall, and weigh 3,500 to 4,500 pounds.
spring/summer/fall, 2016	Installation and maintenance of 1.5 ha of native trees, shrubs and wildflower meadows. Water feature installation (185 Perennials and 47 Shrubs). Two Road garden (432 Perennials, 123 Shrubs/Trees, and 20,000 seed dispersed).	
April – May, 2017		Planted 30,000 *Spartina alterniflora* plugs over a 4,860 m^2 area. 75 native shrubs/trees planted in the upland.
May 2018	Installed a high marsh consisting of 14,000 plugs of *Spartina patens*, 12 anchored tree root wads, and an undulating dune system consisting of 54 mounds of sand planted with native beach grass, Indian grass, switchgrass, big bluestem and seaside golden rod.	

The reference fringing and meadow marshes located in the same estuary were compared to the restored fringing marsh to assess differences in carbon sequestration. Biodiversity indices at the three sites were also compared. We hypothesized that within five years, ecosystem services of the restored fringing marshes would be similar to naturally occurring fringing marshes but less than the meadow marsh. The work conducted under this grant establishes a baseline, as both marshes are less than five years old. We further predicted that seasonal variation in carbon content of the restored marsh would be greater than that of the reference marshes, due to the more dynamic sedimentary environment of the restored marsh, as it matures.

3 Methods

Stations were established at six sites in the mouth of the Housatonic River, CT (Figure 6). The restoration at Stratford Point includes an older section of reef balls and plantings established in 2014 (ID: Stratford Point North, SN) and a newer section of reef balls and plantings established in 2017 (ID: Stratford Point South, SS). Station locations at each site were determined following the methods of Paul et al. (2003),

where a hexagonal grid is overlain on the site map and a randomly generated GPS point within each grid designates the station. The number of stations at each site was based on the size of the marsh area, using the same size hexagonal grid for all sites; four to six stations were sampled per site (Table 2, Figures 7 to 11). Sampling occurred in 2018.

Table 2: Site Characteristics
Site Names correspond to those shown in Figure 6.

site name	marsh type	age of marsh in 2018 (years)	area of marsh (ha)	number of stations
Stratford Point North	restored fringing marsh	4	0.1	4
Stratford Point South	restored fringing marsh	1	1.2	6
Milford Point North	fringing marsh	16 to 20	9.3	4
Milford Point South	fringing marsh			6
Charles Wheeler Marsh North	meadow marsh	>130	223	6
Charles Wheeler Marsh South	fringing marsh			4

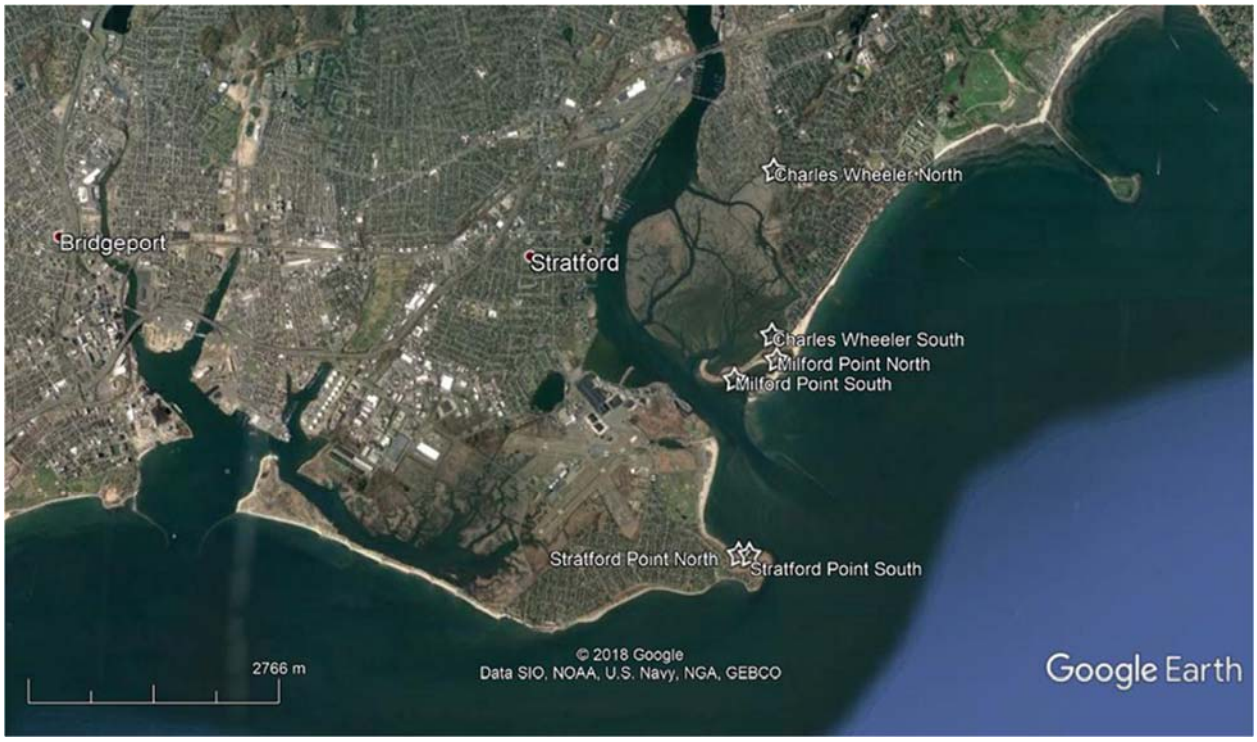

Figure 6: Overview of Sampling Site Locations
Sampling sites are designated by white stars. Stratford Point North and South are the restored fringing marshes. Charles Wheeler North is the meadow marsh. The remaining marshes are established fringing marshes. Four to six stations were sampled per site (Table 2, Figures 7 to 11).

Figure 7: Stations at Stratford Point North and Stratford Point South, Restored Fringing Marsh
The top figure shows the location of stations at Stratford Point North (yellow diamonds, M-P) and Stratford Point South (orange triangles, G-K). The double line of reef balls is visible running roughly parallel to the beach. The Google Earth base image was from 4/22/18, marsh grass has just started to grow. Lower left photo: view of Stratford Point North from just landward of station L, facing west, in October, 2018. Lower right photo: view of Stratford Point South from just landward of station K, facing east, in October, 2018. Note the presence of S. alterniflora *(green, marsh grass) on the inside of the line of reef balls. Additional marsh grass is submerged in these photos.*

Figure 8: Stations at Milford Point North, Fringing Marsh
The Google Earth base image was from 4/22/18, marsh grass has just started to grow.

Figure 9: Stations at Milford Point South, Fringing Marsh
The Google Earth base image was from 4/22/18, marsh grass has just started to grow.

Figure 10: Stations at Charles Wheeler Marsh South, Fringing Marsh
The Google Earth base image was from 4/22/18, marsh grass has just started to grow.

Figure 11: Stations at Charles Wheeler Marsh North, Meadow Marsh
The Google Earth base image was from 4/22/18, marsh grass has just started to grow.

3.1 Carbon Sequestration Rate

Carbon sequestration was measured as a function of sediment accumulation rate and sediment carbon density, which is calculated from soil bulk density (g cm^{-3}) and percent carbon in soil (Chmura et al. 2003; Craft et al. 1991).

Temperature and light sensors (Onset HOBO Pendent, 64k) were deployed at each of the thirty stations throughout the study period. These data were used to assess contributing factors to variability in carbon sequestration rates. Combined, the temperature and light data also indicate timing of inundation at a station.

3.1.1 Sediment Accumulation Rate

At each station (30 total), short-term (1-year) sediment accumulation rates were determined using a marker horizon (e.g. clay-feldspar) following the methods of Lynch et al. (2015, SOP 5). Each station was marked with a rebar stake and the clay-feldspar was placed on the ground a known distance to the north of the stake (Figure 12). The clay-feldspar was deployed in mid-April 2018 and resampled on November 20, 2018. Two plugs were cut from each marker horizon and distance was measured with calipers; if the marker horizon could not be found, at least three attempts were made within the target area. We assumed that accretion rate between November 2018 and April 2019 would be similar to the rate of accretion between April 2018 and November 2018 and adjusted the measured accretion rates to an annual value. Future work will confirm this assumption by resampling the marker horizons in November 2019.

Figure 12: Establishing a Station Each station included a rebar stake with HOBO light and temperature logger with a marker horizon of clay – feldspar a known distance north of the rebar stake. Shown: Charles Wheeler North, station A.

Stratford Point North and South were dynamic environments and the marker horizons had been washed away within a few weeks; these sites require sediment plates to establish sediment accretion rates. In lieu of marker horizons, we estimated sediment accretion rate based on observed sediment accumulation at our station markers and included a very generous estimate of potential error. In 2019, sediment plates were deployed at each station to assess accretion rate for future work at these sites. Sediment accretion rates from the plates deployed in 2019 will replace our rough estimate of sediment accretion rates presented in this report and carbon sequestration rates presented here for SN and SS will be recalculated with the new accretion data.

3.1.2 Sediment Bulk Density and Carbon Content

For each sampling event, sediment was collected from three areas at each station and composited in the field for analysis. At Charles Wheeler North and Charles Wheeler South, where the marsh is older and

has developed a peat layer, a 5 cm by 40 cm core was collected with a Russian Peat Borer (USEPA 1999). Each core was divided into 10 cm sections in the field (Figure 13). At all other sites, sediment samples were obtained by digging out a known volume from the top 5 cm of sediment and a second sample for the sediment from 5 to 10 cm of depth; these sediments were rocky and often water-logged.

Figure 13: Sediment Cores Three cores collected from a station and divided into 10 cm sections; the upper most layer (0-10cm) is at the top and the deepest section (30-40cm) is at the bottom.

Each section of each of three cores per station was divided in the field into three bags destined for three separate analyses. The Standard Operating Procedure in Appendix A provides details on the analytical procedures, a summary is provided here:

- One sample was rinsed with water over a mesh screen to remove sediment and obtain the plant weight as a fraction of the initial wet weight; plants were then dried at 50°C to obtain dry weight and saved for future analyses not included in this project.
- A second sample was used to determine bulk density (g-sediment cm^{-3}) and the carbon content (%C) via the loss-on-ignition technique. These samples were weighed for wet weight, dried at 50°C and weighed (for comparison to plant fraction), dried at 105 °C and weighed, homogenized and large clumps were broken apart, then muffled at 550 °C for 4.5 hours (started in cold oven) and weighed.
- The third set of samples was processed for elemental analysis. Samples were weighed for wet weight, dried at 50°C and weighed (for comparison to plant fraction), dried at 105 °C, ground to finer than 2 mm and stored in acid-washed glass scintillation vials until analysis for carbon and nitrogen on a Fisons NA 1500 Series 2 Elemental Analyzer (Costech Analytical Technologies, Inc.). These samples were tested for carbonates prior to elemental analysis by exposing ~1 cm^3 of the sample to 6 N hydrochloric acid and observing for effervescence; carbonates, which are inorganic carbon, can confound the elemental analysis of organic carbon. Only two samples contained carbonates and were treated with 6 N hydrochloric acid prior to further analysis; both samples were from the Stratford Point south site, station G, the 0 to 5 cm depth and the 5 to 10 cm depth (Figure 7, page 11).

Carbon sequestration (g m^{-2} y^{-1}) was estimated following the methods of Chmura et al. (2003), with soil organic carbon (%C) determined from soil organic content (% organics) via loss on ignition of sediment (LOI, % organics), and validation by elemental analysis of a subset of soil samples for organic carbon (%C), following the methods of Craft et al. (1991):

$$\text{Organic C} = 0.40\ \text{LOI} + 0.0025\ \text{LOI}^2$$

To verify the use of the conversion from loss-on-ignition determination of organic content of sediment to organic carbon in sediment developed by Craft et al. (1991), the June sediment samples were also analyzed on an elemental analyzer to directly measure organic carbon content. All depth intervals were included in the analysis, not just the surface layer. The LOI technique and conversion to carbon content

underestimates the %C by about 14% (Figure 14). An analysis of the impact of this error on final carbon sequestration rates indicated that the carbon sequestration rates were underestimated by ~25%. Because of this, the directly measured carbon content was used in calculating carbon sequestration rates, when available. For samples with only LOI data, the %C was converted using the equation shown in Figure 14.

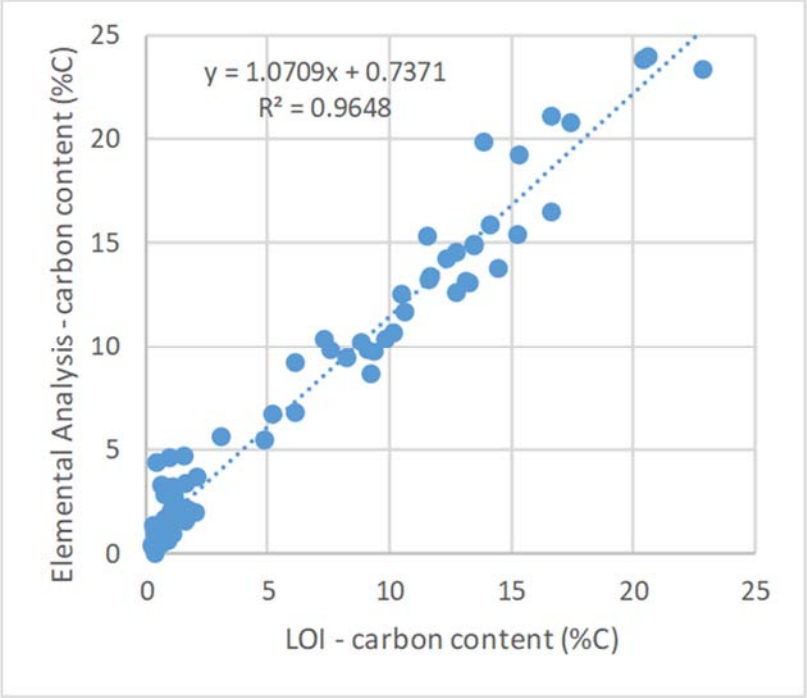

Figure 14: Comparison of Carbon Content from Loss-on-Ignition and Direct Measurement
The June sediment samples for all depths were analyzed with both the loss-on-ignition method and via direct measurement on an elemental analyzer. The dotted line is the regression line of these data.

3.1.3 Timing of Sediment Sampling

All stations were sampled in June of 2018 (within three days of 6/20/18) to assess the average and spatial variability within each site. Two stations within each site were sampled on four dates between April and October of 2018 to assess the temporal variability – to see if the date of sampling impacts our estimate of carbon sequestration rate. Stations were sampled within three days of the date listed: 4/13/18, 6/20/18, 8/8/18, 10/20/18.

3.1.4 Sediment Data Analysis

For the sediment data, cores were averaged across the marsh by depth, yielding an average value for each depth sampled. The bulk carbon density averaged across the whole core (all depths) was used for the calculation of carbon sequestration, this is the standard protocol for this method.

A one-way repeated measures ANOVA and appropriate all pairwise multiple comparisons procedures were used to compare carbon sequestration rate among marshes using *SAS JMP 13.0* or *SPSS SigmaPlot 14.0*. Data transformations or non-parametric alternatives were used as necessary.

3.2 Vegetation Surveys

In November 2018, the above-ground plant community at each station was assessed for species present, the percent cover of each species, and stem counts of *Spartina alterniflora* and *Spartina patens*. Percent cover was assessed in the field and species were identified within a circular quadrat of 0.83 m^2. A plan-view photograph of the quadrat was taken before counting stems. The photograph was later analyzed in the lab by overlaying a 100-box grid with boxes of equal sizes, using the grid conformation of Beckers and Beckers (2012). The lab-analyzed photo results were used for data analysis and the field estimate of percent cover was referenced for confirmation of species identification and as validation of the lab analysis.

3.3 Comparison of Vertebrate Use of Reef Balls Versus the Fringing Marsh

3.3.1 Fish Sampling

Fish utilization of the natural fringing marsh at Milford Point North and Milford Point South were compared to Stratford Point by trapping fish in baited minnow traps. Video cameras were deployed at Milford Point South and Stratford Point to get a better understanding of fish utilization of the reef over time using a non-invasive technique.

In June, July, August, and October, twelve minnow traps baited with dog food, squid, and bread were deployed at Milford Point North, Milford Point South and Stratford Point. The choice to bait the traps was made by comparing the community caught by an unbaited trap relative to a community caught by baited traps. The test was conducted at Bakers Pond marsh in Groton, CT. Results indicated using all three bait types would draw a species assemblage similar to that of the unbaited traps and maximize the number of fish attracted to the minnow trap.

Four traps were deployed per site. For this comparison Stratford Point North and Stratford Point South were combined into one site. Traps were deployed approximately three hours before high tide and retrieved approximately three hours after high tide. Due to variations in processing time for collected organisms, traps were deployed for between 5.5 and 8 hours. Upon retrieval, the animals in the trap were weighed and fish were separated from invertebrates. Invertebrates were identified, counted, measured, and released. The remaining contents, fish, were weighed separately, rapidly sorted into three size classes, individuals were counted and identified to the lowest practical taxonomic unit (genus and species where able), and then released. Traps were deployed on the same day for all sampling events except July, where traps were deployed at Stratford Point two days after the initial sampling.

GoPro video camera observations were made in alternate months from the trap surveys: May, July, and September. Four cameras were placed inside different reef balls along the shoreline as the tide was coming in at Stratford Point. Within one day, the same four cameras were moved to Milford Point and deployed at the edge of the marsh grass closest to the open water. Cameras were positioned on the bottom, facing the surface of the water. The cameras were retrieved during low tide. We tried to limit camera days to calm, cloudless days and high tide cycles during noon for maximum lighting. The September sampling at Stratford Point was conducted on a stormy day with high wave action and low visibility; due to timing, we were not able to re-sample this site on a better day and data were excluded from the analysis.

Video cameras were deployed for 50 to 100 minutes at a site, four per site. Video were analyzed by watching the video and noting which species were present, when they appeared on the screen and when they left the screen. The number of individuals of each species on the screen was also tracked. The full length of the video was analyzed in most cases. For videos with many individuals, at least one 16-minute section was analyzed. If the 16-minute section yielded a count greater than 200 individuals, only that length of video was analyzed. In cases where the observation of 200 individuals was achieved in less than 16 minutes, the full 16-minute length was still analyzed. In cases where only parts of the video were analyzed, Vaudrey reviewed all video sections, watching 10% of the video to confirm that the 16-minute section reviewed was not anomalous relative to the other three to four sections of video recorded for that station.

3.3.2 Calculation of Diversity Indices

Data on vertebrate species and counts from the video observation were used in a variety of diversity indices: the sequential comparison diversity index, the Shannon diversity index, and Simpson's inverse dominance index (Brower et al. 1990). The Shannon index is most affected by the occurrence of rare species while Simpson's dominance index is most sensitive to the relative abundance of species; using both indices captures both impacts on diversity. The video data provides a random sample of the community and so is appropriate for calculating diversity; whereas, the minnow traps were baited and limited to smaller animals and are thus a non-random sampling. It should be noted from the outset that we expect salt marsh diversity to be very low compared to other aquatic and terrestrial communities where these indices are typically employed (Keddy 2000).

The sequential comparison diversity index (*SCI-DI*) considers the species richness (*R*, number of taxa) and how frequently the sampled organism is the same species as the previous organism (Brower et al. 1990). To calculate, "runs" of species are recorded, where a run is a series of individuals of the same species observed in the video. In the following example, there are nine runs with fourteen individuals, where letters indicate species and the underlined sections constitute a run:

A B B A C C C B A A B C C D.

The sequential comparison index (SCI) is calculated as:

$$SCI = \frac{number\ of\ runs}{n} \qquad \textit{(Equation 1),}$$

where *n* is the number of individuals examined. The greater the diversity of organisms, the higher the SCI; which ranges from a low diversity of 1/*n* to a high diversity of 1. The SCI can be used to calculate a more refined estimate of diversity by multiplying by the species richness (*R*):

$$SCI\text{-}DI = SCI \cdot R \qquad \textit{(Equation 2),}$$

Streams typically have values ranging from 8 to greater than 12 (Brower et al. 1990), though we expect the values for tidal marshes to be considerably lower (Keddy 2000).

Shannon's diversity index (*H'*) considers the proportion of the total number of individuals occurring in a species (Brower et al. 1990). This index is based on the concept of uncertainty - how well are you able to predict the identity of the next individual sampled. This index works well in sampling schemes involving taking samples at random from a community, as in the video sampling. It should not be used for

selective sampling techniques such as traps, seines, artificial substrate, and laboratory manipulations. The index was calculated as:

$$H' = \sum_{i=1}^{R} \frac{n_i}{N} \cdot ln \frac{n_i}{N} \qquad \textit{(Equation 3),}$$

where *N* is the total number of individuals, *n* is the number of individuals of a species, and *R* is the species richness (total number of taxa observed). Values range from zero to five, with zero being the lowest diversity. Across all communities (terrestrial and aquatic), values typically fall in the 1.5 to 3.5 region.

Simpson's inverse dominance index (1/λ) considers the number of species (*R*), total number of individuals (*N*) and the proportion of the total that occurs in each species (Brower et al. 1990). Individuals were not removed from the population by sampling, so the simplification of the Simpson's Dominance Index (λ) was used, where dominance is the probability that two individuals will belong to the same species. A highly diverse community will have low dominance. To get at true diversity, ecologists take the inverse of λ; which is an expression indicating the number of times you would need to take pairs of animals from the community before finding two of the same species. This modification of the index is especially useful when diversities are low and similar, allowing for the differences between two samples to become more apparent. The index was calculated as:

$$\frac{1}{\lambda} = \frac{N^2}{\sum_{i=1}^{R} {n_i}^2} \qquad \textit{(Equation 4),}$$

where *N* is the total number of individuals, *n* is the number of individuals of a species, and *R* is the species richness (total number of taxa observed). Values range from one to infinity, with one being the lowest diversity.

3.3.3 Bird Sampling

Our original plan included establishing a blind and observing bird use of the reef balls and marsh at Stratford Point versus observations made at Milford Point during May and September. This plan proved to be overly ambitious. The observed bird use was minimal during the times of observation, especially as we were looking for birds using the shoreline, versus those passing by or in distant areas. To accurately assess bird use, we would need to spend a great deal more time making observations. For this reason, the bird data are not included in this report.

4 Results

4.1 Sediment Accumulation Rates

Sediment accumulation rates were estimated by marker horizon at the established marsh sites (CN, CS, MN, MS). At the Stratford Point sites (SN, SS), the sediment accumulation rate is a very rough estimate based on observations of sediment accumulation at the station marker coupled with a generous error estimate. These sediment accumulation rates will be refined in future years; sediment plates have already been deployed at these sites and estimates should be available in 2020.

4.1.1 Sedimentation Rate at Stratford Point North and South, Estimated

The accretion rate was estimated based on examining the location of the deployed HOBO temperature sensors relative to the sediment surface at the end of the deployment. At the start of the deployment, the sensors were deployed level with the surface of the sediment. The accretion rate varied widely across the stations and in some cases, was negative, indicating sediment loss. To obtain a single value of accretion for each site, the average accumulation rate for all stations in a site (SN or SS) were averaged together. The error was estimated from ranges of sediment accretion from the literature; these ranges are not based on accurate values for this site, but are first-cut estimates of the potential range of accretion. Once we have data, we expect the range of the accretion rates to be smaller and better constrained. This process yielded an estimated accretion rate of 1.5 cm y^{-1} for Stratford Point North (4 years post reef ball installation) with a range of -1.95 to 4.91 cm y^{-1}, and an accretion rate of 0.10 cm y^{-1} for Stratford Point South (1-year post reef ball installation) with a range of -2.88 to 2.88 cm y^{-1}.

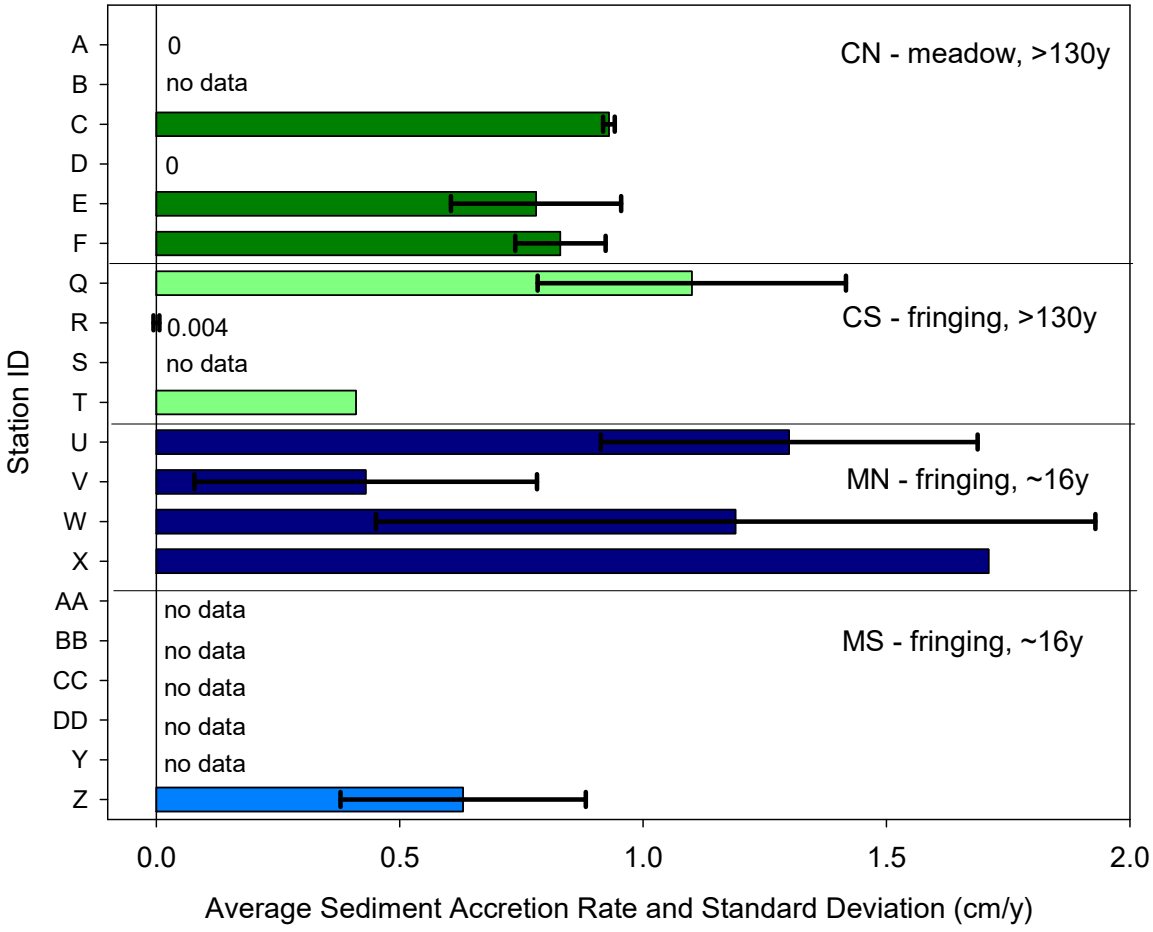

Figure 15: Sediment Accretion Rates by Station
Horizontal lines and colors of bars divide the stations into the four sites. Accretion rate was estimated by marker horizon. Error bars for these sites are the standard deviation of two replicate measurements. For stations with no bar showing, information is provided on the rate, "no data" indicates the marker horizon could not be found (this does not necessarily imply sediment loss as other factors impact stability of the marker horizon over time, including bioturbation).

4.1.2 Sedimentation Rate at Established Marshes, via Marker Horizon

The accretion rate of these older marshes (16-years old, >130 years old) tended to be similar within a site (Figure 15). To obtain a single value of accretion for each site, the average accumulation rate for all stations in a site were averaged together. For use in statistical comparisons, the standard deviation for a site was calculated from the standard deviation of each station using propagation of error. These results were used in a one-sample t-test for each site at the $\alpha = 0.05$ level. The Milford Point South site (MS) was not included in these statistical analyses because only one station had accretion data; MS was a rocky site characterized by many bivalves, rocks, and seaweed among the plant roots (Figure 16). For all other sites, the sample mean of the group exceeded the hypothesized mean (of 0) by an amount that is greater than would be expected by chance, rejecting the hypothesis that the hypothesized mean (of 0) is greater than or equal to the true mean: CN one-sample $t(3) = 25.74$, $p < 0.001$; CS one-sample $t(2) = 8.365$, $p = 0.007$, MN one-sample $t(3) = 10.25$, $p < 0.001$.

The minimum accumulation rate and maximum accumulation rate were calculated as two standard deviations for all stations within a site, to use in the calculation of carbon sequestration.

Figure 16: Photo of a Milford Point South (MS) Station
The presence of mollusks likely contributed to the loss of the marker horizon at the stations in this site, due to excessive bioturbation and sediment movement.

4.1.3 Sediment Accumulation Summary

A one-way ANOVA on Ranks comparing the site-wide average accumulation among all sites (and standard deviation as calculated in Section 4.1.1 and 4.1.2) indicated no statistically significant differences among the sites at $\alpha = 0.05$ ($H(5) = 5.173$, $p = 0.395$).

Given the large variability within sites, the average accumulation rate with two standard deviations from the mean used as the minimum and maximum accumulation rates were used for the calculation of carbon sequestration (Figure 17).

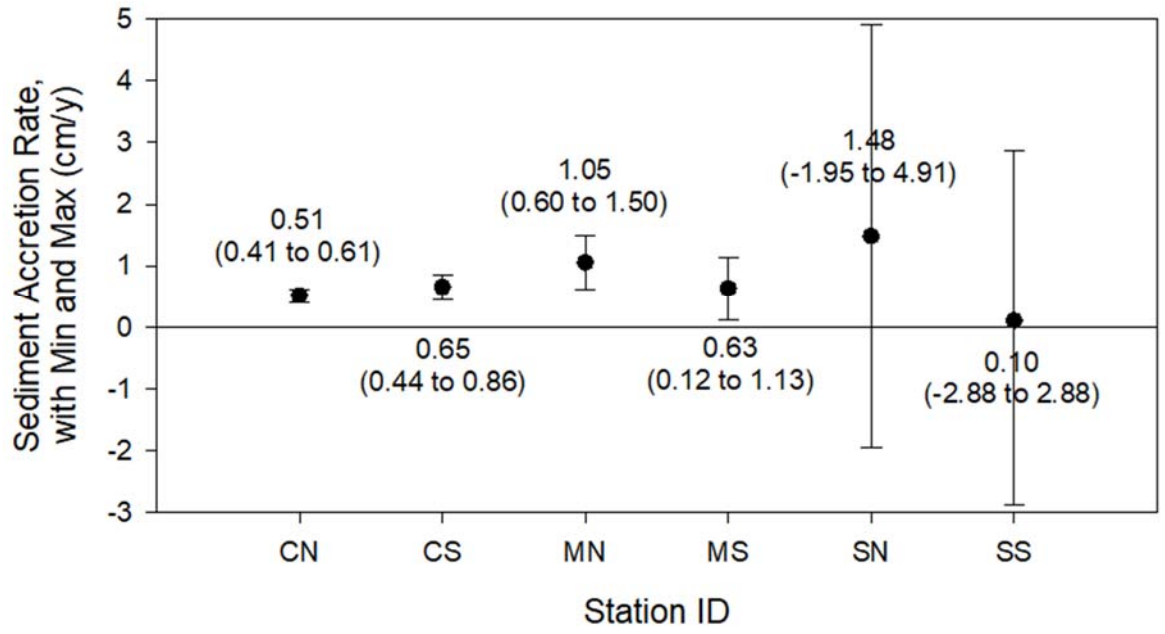

Figure 17: Sediment Accumulation Rates by Site
Average sediment accumulation was calculated from marker horizon data (CN, CS, MN, MS) or a rough estimate is provided (SN, SS). Given the large variability within sites, the average accumulation rate and the minimum and maximum accumulation rates estimated as two standard deviations from the mean were used for the calculation of carbon sequestration (error bars).

4.2 Sediment Characteristics

Station locations are provided in Appendix B (page 52). All stations were sampled in June of 2018 to assess the spatial variability of sediment characteristics and obtain an average value for each site (Figure 18). Two stations in each site were assessed four times between April 2018 and October 2018, to assess temporal variability in sediment characteristics (Figure 19).

Spatially, sediment bulk density (g cm^3) was tightly constrained for the Charles Wheeler Marsh sites (CN, CS) and showed greater variability across the stations in the other sites (MN, MS, SN, SS) (Figure 18). The Charles Wheeler sites had a bulk density around 0.12 g cm^3 while the other sites had a higher bulk density around 0.45 g cm^3, except for MS which had a high bulk density of 0.75 g cm^3. Seasonally, sediment bulk density was similar across all dates for the Charles Wheeler Marsh sites (CN, CS), but showed higher bulk density in October for the other sites (MN, MS, SN, SS) (Figure 19).

The fraction of organic carbon in sediment is multiplied by the bulk density to obtain the carbon content of the sediment (Figures 18 & 19). The spatial sampling indicates that carbon content of the top layer of soil was higher for the Charles Wheeler Marsh sites (CN, CS) than other sites, with an average carbon value of 0.014 g m^{-3} in both sites (Figure 18). The two established fringing marshes at Milford Point had lower values, with MN carbon at 0.005 g m^{-3} and MS carbon at 0.009 g m^{-3}. The recently restored marshes at Stratford showed the lowest carbon content with the 4-year old SN at 0.003 g m^{-3} and the 1-year old SS at 0.002 g m^{-3}. The temporal sampling indicates that sediment carbon content generally increased between April and October, consistent with the growth of plants and algae through the summer months, though the carbon content of sediment from the recently restored marshes was consistently low throughout the sampling period (Figure 19).

The sediment accretion rate was covered in Section 4.1 (page 19). Sediment accretion rate is multiplied by the sediment carbon content to estimate the carbon sequestration rate. The values for the surface layer for each station and date are provided in Figures 18 & 19, for evaluating what factors impact variability in carbon sequestration. An average sediment accretion rate is derived for each site and applied throughout the year, thus there is no variability among stations or among sample dates. The sediment accretion rate does have a large error associated with the estimate; the error bars shown in the figures are the minimum and maximum estimated accretion rates, calculated as two standard deviations from the mean. These are used to calculate the minimum and maximum carbon sequestration rates.

Given that sediment accretion rate is steady across a site and across sample dates, the variability among stations (Figure 18) and sampling dates (Figure 19) seen in the carbon sequestration rates are a result of variations in the carbon content of the sediment. The range on the carbon sequestration rates at the newly restored marshes at Stratford Point are considerably greater than the other sites because the sediment accumulation rate is a rough estimate with a generous range of error applied. Future work in Stratford will include deployment of sediment plates to better estimate accretion rates.

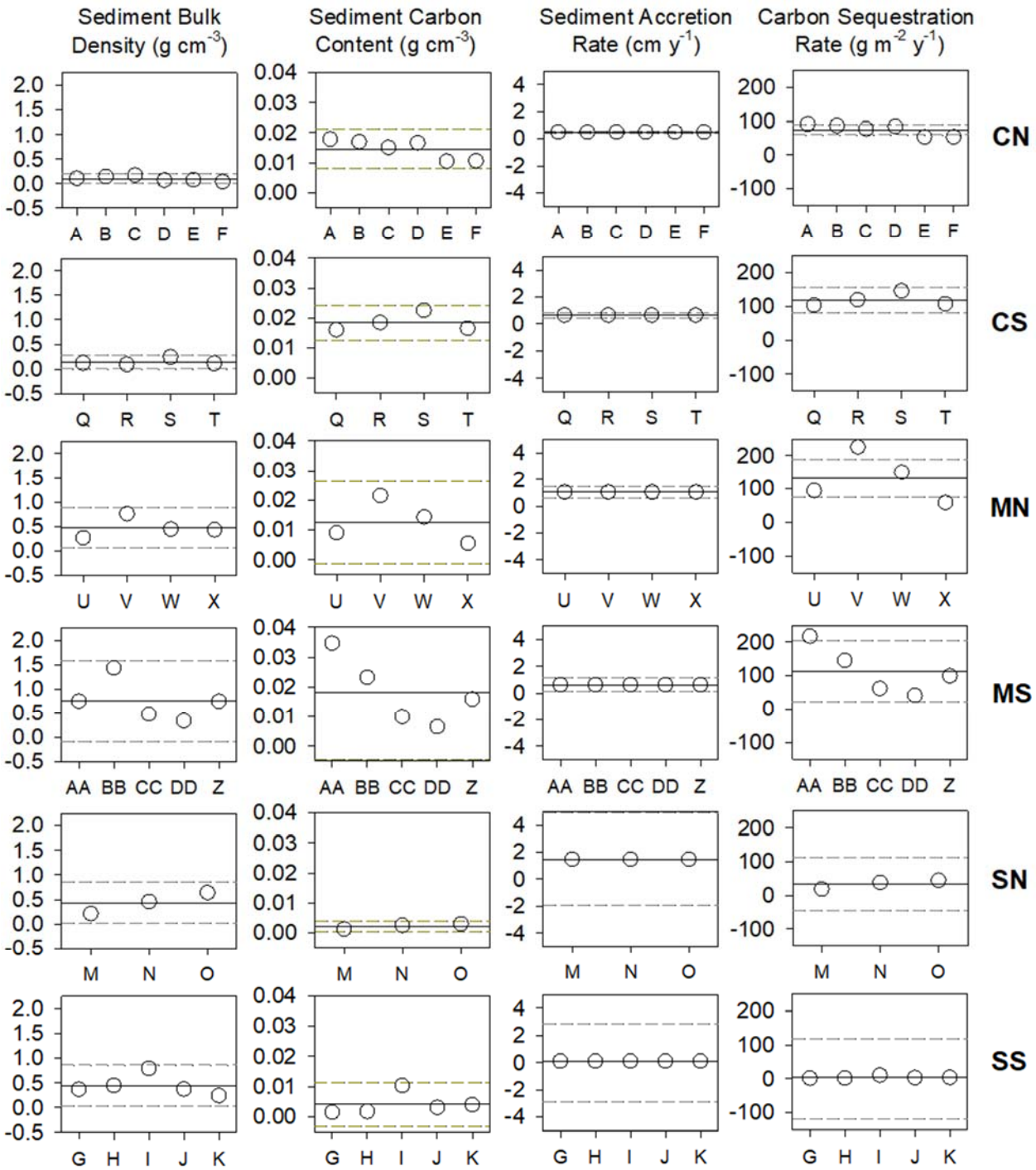

Figure 18: June Sampling for Spatial Averages and Variability
Data for each station in each site are indicated by the symbols; site codes are listed to the right of the figure and the station letters are on the x-axes. The solid lines are the mean of all stations for the sites for the surface layer only. For sediment bulk density and sediment carbon content, the dashed lines are two standard deviations from the mean. For sediment accretion rate and carbon sequestration rate, the dashed lines are the average of the min and max values of all stations for the site, for the surface layer.

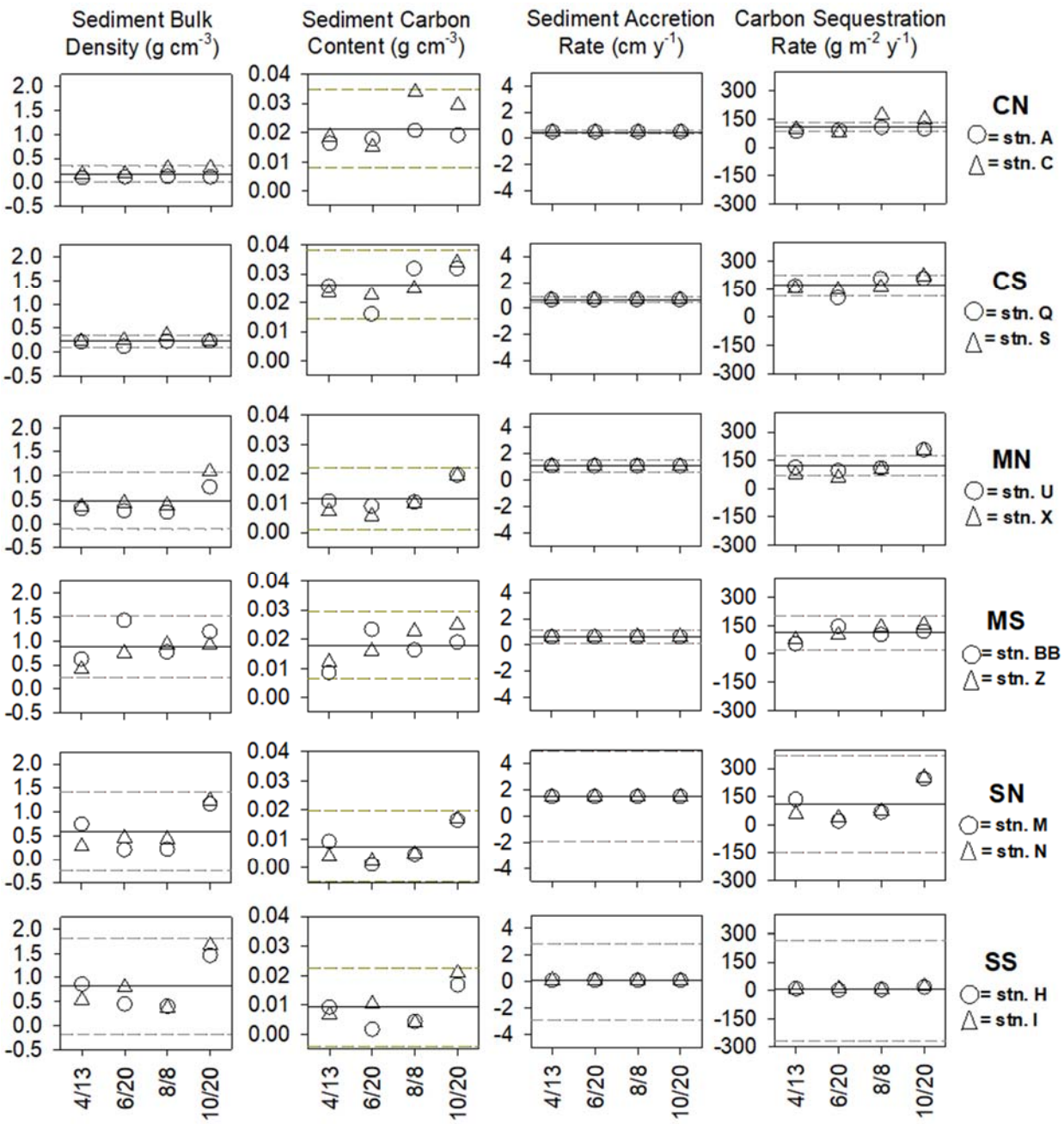

Figure 19: Sampling of Two Stations per Site to Assess Seasonal Variability
Data for each station in each site are indicated by the symbols; site codes and station identifiers are listed to the right of the figure and the sample dates are on the x-axes. The solid lines are the mean of all stations for the sites, for the surface layer only. For sediment bulk density and sediment carbon content, the dashed lines are two standard deviations from the mean. For sediment accretion rate and carbon sequestration rate, the dashed lines are the average of the min and max values of all stations for the site, for the surface layer.

4.3 Carbon Sequestration Rates

The averages of the carbon bulk density for all depth layers sampled were used in the calculation of the carbon sequestration rate. Carbon bulk density varies with depth in the sediment, even in established marshes (Adame et al. 2013; Artigas et al. 2015; Nahlik and Fennessy 2016). In the literature, the depth over which carbon bulk density is determined varies, ranging from 10 cm to 200 cm, but typically in the 30 to 100 cm range. Carbon stock (ton ha^{-1}) is reported for our data, but these values should be considered a minimum estimate of the carbon stock because we sampled only the surface 40 cm in established marshes to obtain a carbon sequestration rate representative of the recent past (< 200 years) (Figure 20). As a marsh ages and builds up peat, the carbon stock will also increase.

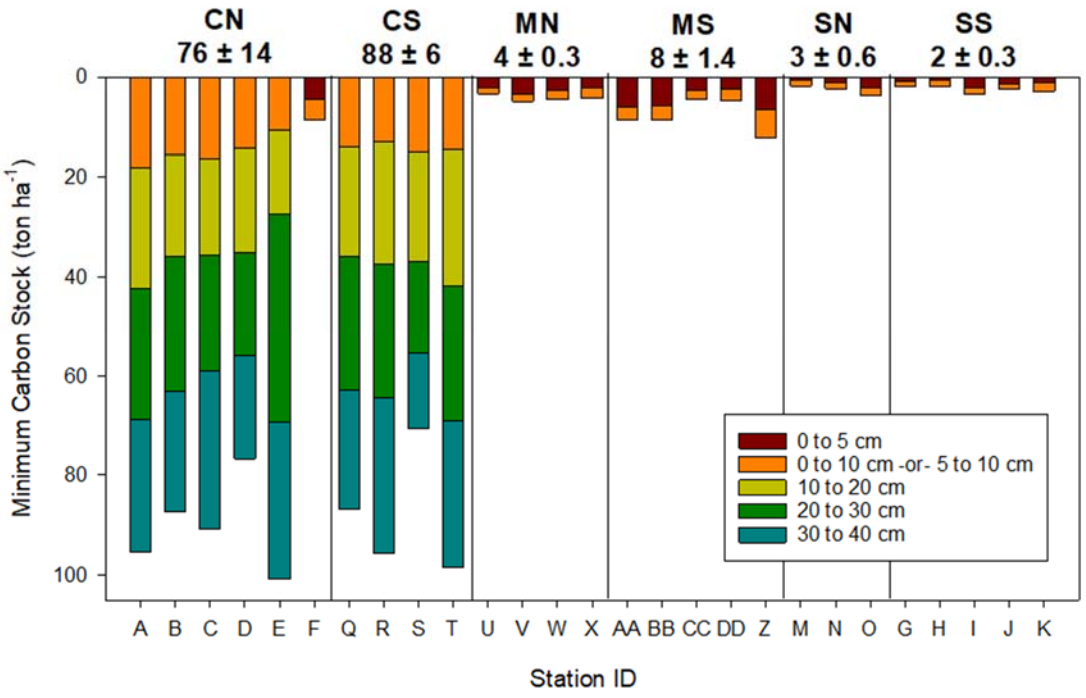

Figure 20: Minimum Carbon Stock
Carbon stock for each station sampled in June; the average and standard error for each site are listed above the figure. This is considered a minimum because only the upper 40 cm was sampled. In the fringing marshes, the sediment was rocky and water-logged; samples were only collected to 10 cm depth in these cases. For both the Milford and the Stratford sites, a deep marsh peat layer has not developed; deeper sediment are not representative of marsh accretion and were not collected. (ton = 10^6 g = Mg)

The carbon sequestration rates were averaged across each site (Figures 18 & 19), to yield an estimate of the sequestration rate for each marsh, with the standard error representing the variability within a site (Figures 21 & 22).

The June sampling revealed statistically significant differences among the carbon sequestration rates of the sites (Figure 21) (one-way ANOVA and Holm-Sidak multiple comparisons procedure; $F(5,21) = 14.535$, $p < 0.001$). The average for established fringing marshes in this study ranges from 90 to 180 g m^{-2} y^{-1} (CS, MN, MS); the 4-year old restored marsh (SN) average is lower (~25 g m^{-2} y^{-1}) but statistically similar to both the rockier established fringing marsh and the 1-year old restored marsh (SS) (Figure 21). The marsh meadow at Charles Wheeler north (CN) had a carbon sequestration rate of around 110 g m^{-2} y^{-1}, within the range of established fringing marshes.

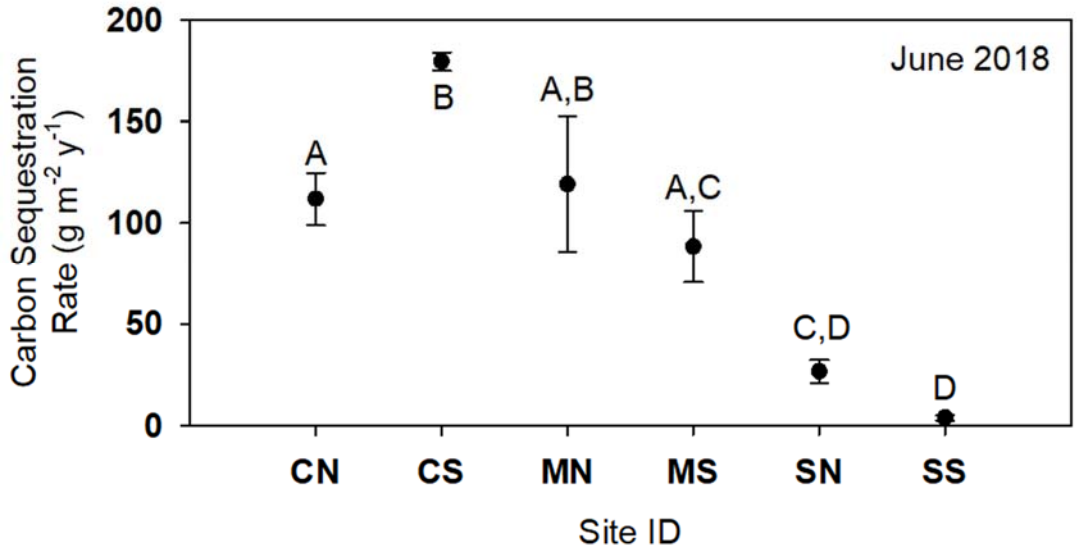

Figure 21: Comparison of June Carbon Sequestration Rates Among Sites
Points represent the average carbon sequestration rates in each site. The number of stations included in each average were: CN = 6, CS = 4, MN = 4, MS = 5, SN = 3, SS = 5. The error bars are the standard error. Letters indicate statistical similarity based on a one-way ANOVA and Holm-Sidak multiple comparisons procedure ($F(5,21) = 14.535$, $p < 0.001$).

The temporal sampling of two stations per site indicates that differences in carbon sequestration are seen based on when you sample during the year (Figure 22). Statistical similarity was evaluated for all sites independently using a one-way ANOVA and a Holm-Sidak all pairwise multiple comparison test with an $\alpha = 0.05$. No statistically significant differences were seen among sampling dates for the marsh meadow at Charles Wheeler North (CN) ($F(3,4) = 0.849$, $p = 0.535$). Three of the fringing marshes exhibited a statistically significant difference among sample dates, but the difference was weak enough that a Holm-Sidak multiple comparison procedure indicated that all pairwise comparisons were not significant at an $\alpha = 0.05$; these included the oldest fringing marsh at Charles Wheeler South (CS; $F(3,4) = 7.314$, $p = 0.042$), the fringing marsh at Milford Point South (MS; $F(3,4) = 8.081$, $p = 0.036$), and the youngest restored marsh at Stratford Point South (SS; $F(3,4) = 8.326$, $p = 0.034$). For the 4-year old restored fringing marsh at Stratford Point North (SN) and the established fringing marsh at Milford Point North (MN), October was statistically significantly different from other dates. For MN, October was different from all dates, with all other dates being statistically similar ($F(3,4) = 42.635$, $p = 0.002$) and for SN, October was different from June, with all other comparisons being similar ($F(3,4) = 11.944$, $p =$

0.018). Based on these results, sampling in October is likely to overestimate carbon sequestration rates, while sampling at any time earlier in the year (April through August) should not adversely bias the estimates of carbon sequestration rates.

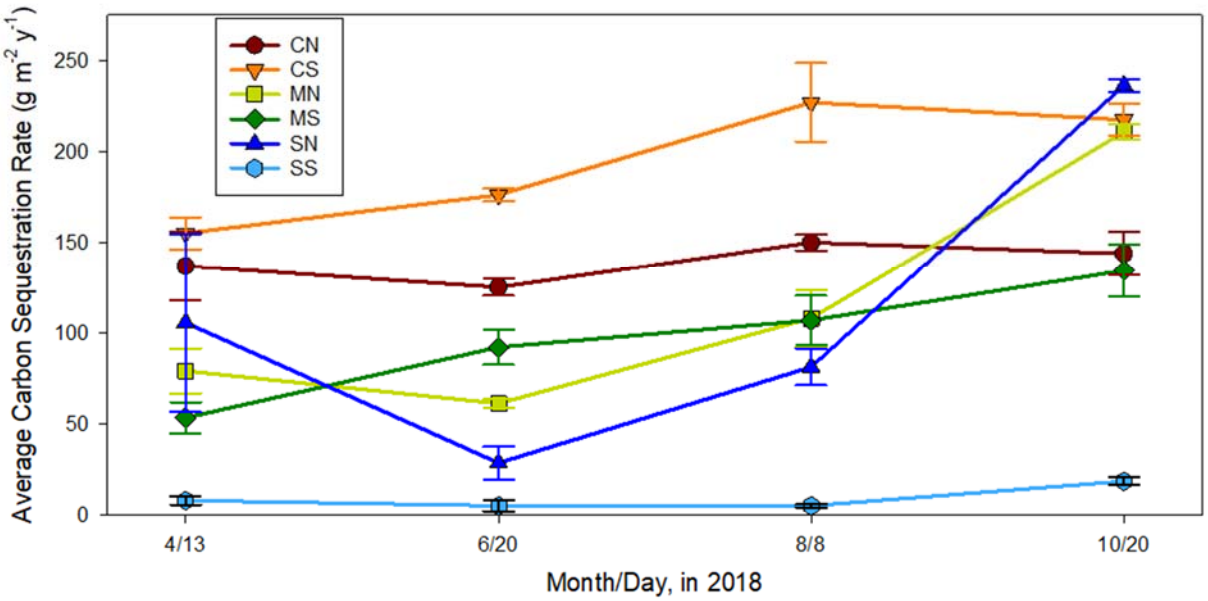

Figure 22: Comparison of Carbon Sequestration Rates Over a Growing Season
Points represent the average carbon sequestration rates in each site for each sampling date. Two stations were included in each average; the error bars are the standard errors.

Carbon sequestration rates are dependent on the carbon content of the sediment, which in turn is theoretically enhanced by greater plant biomass. The presence of plants results in below ground plant biomass (roots and rhizomes) which contribute carbon to the sediment. In November of 2018, each station was assessed for percent cover by plant species and the plants were identified to the lowest practical taxonomic unit. *Spartina alterniflora* and bare sediment accounted for the majority of the cover. One station at Charles Wheeler North (CN), the meadow marsh, was dominated by *Spartina patens*. Additional species found at some stations included:

- *Bolboschoenus* sp. (bulrush, formerly *Scirpus robustus* Pursh) – could have been *Bolboschoenus novae-angliae* (Britton) S.G. Sm. (New England bulrush) or *Bolboschoenus robustus* (Pursh) SojáK (sturdy bulrush)
- *Gracilaria* sp. (graceful red weed, red algae)
- *Ulva* sp., blade form (green sea lettuce, green algae)
- *Blidingia* sp. (green algae)
- *Fucus* sp. (bladder wrack, brown algae)

In general, Charles Wheeler North (CN), the meadow marsh, had the greatest percent cover of *Spartina* sp. (60 to 100%) and higher carbon sequestration rates (Figure 23). The percent cover by *Spartina* sp. for

the older natural fringing marshes (CS, MN, MS) and the older restored fringing marsh (SS) ranged from 20% to 100%. The Milford Point North site (MN) and Charles Wheeler South (CS) generally had the largest sequestration rates in this group while Stratford Point North (SN) had the lowest, though the carbon sequestration rates among sites in this groups were highly variable. The youngest restored fringing marsh (SS) had the lowest percent cover by *Spartina* sp., ranging from 0 to 20% and also had very low carbon sequestration rates, as evidenced by the small bubble sizes in Figure 23.

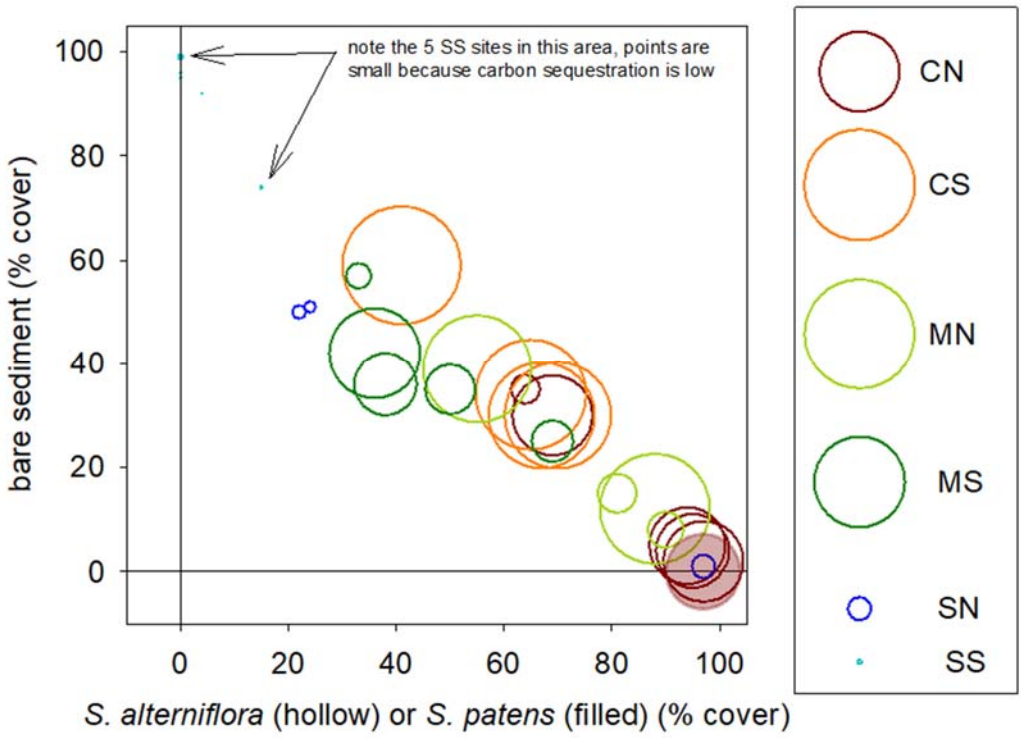

Figure 23: Comparison of Plant Community and Carbon Sequestration by Site
The size of the bubble represents the relative magnitude of carbon sequestration at each site, calculated as a fraction of the largest measured rate. The colors of the bubbles correspond to the site, as defined in the legend; the bubble sizes in the legend are equivalent to the largest bubble shown in the plot for that site. Spartina alterniflora *was the dominant vegetation at all but one site, though other vegetation types were also present. One site was dominated by* Spartina patens*, indicated by the filled red circle.*

4.4 Fish Use

To compare the utilization of the natural fringing marsh to the restored living shoreline, minnow traps were deployed to estimate biomass and species present (Table 3, Figure 24). Only four species of fish were caught in the minnow traps (Table 3), with mummichogs (*Fundulus heteroclitus*) being the dominant species for all sampling dates.

Data show a higher biomass in August and October relative to June and July. However, a sample size analysis to determine the necessary number of samples needed for statistical significance at a desired power of 0.8 and an α of 0.05 indicates a minimum of 112 traps would need to be deployed among the

three sites. Thus, these data provide an estimate of patterns among the sites but cannot be reviewed statistically. Milford Point North, the innermost site at Milford Point, hints at greater biomass than the other two sites in August and October.

Table 3: Species Caught in Minnow Traps
Only four species of fish were caught in the minnow traps. The fraction of the total catch is shown by sampling event, by site, where MN is Milford Point North, MS is Milford Point South, and SP is Stratford Point. The total number of fish caught over the full deployment time is shown in the bottom row; traps were deployed for slightly different length of time and are not normalized for time deployed.

Species Name	Common Name	Fraction of Total Catch (%)											
		June			July			August			October		
		MN	MS	S	MN	MS	S	MN	MS	S	MN	MS	S
Fundulus heteroclitus	mummichog	100	100	100	100	100	100	99.6	99.7	99.3	99.4	74.8	60.1
Fundulus majalis	striped killifish	0	0	0	0	0	0	0.4	0.3	0.7	0.3	0	17.4
Menidia menidia	silverside	0	0	0	0	0	0	0	0	0	0	24.9	22.0
Centropristis striata	black sea bass	0	0	0	0	0	0	0	0	0	0	0.2	0.5
Total number of fish		***39***	***1***	***131***	***227***	***7***	***148***	***1162***	***755***	***294***	***1328***	***503***	***218***

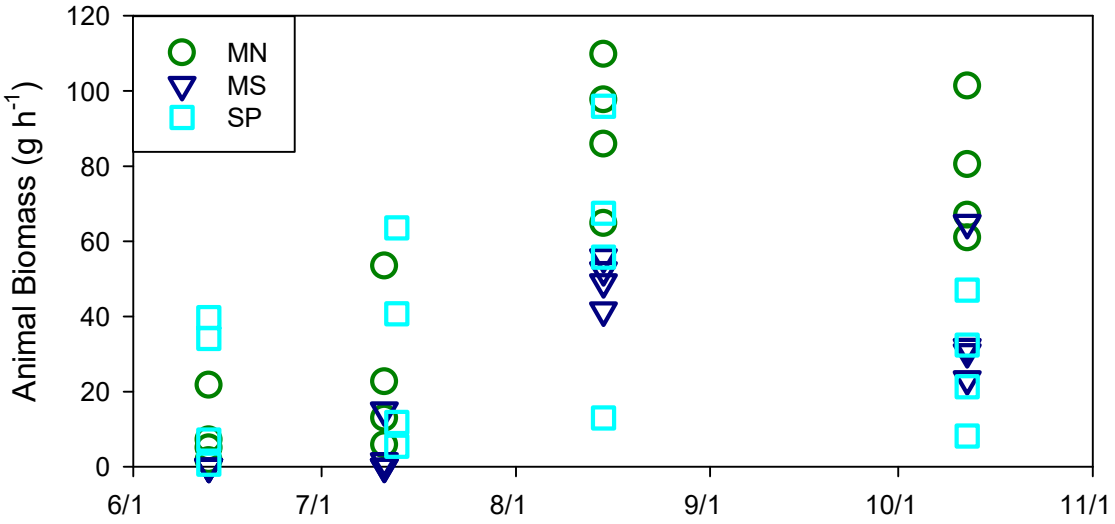

Figure 24: Biomass Caught in Minnow Traps
Each point represents the average biomass caught in a trap per hour of deployment. MN is Milford Point North, MS is Milford Point South, and SP is Stratford Point.

The second method for assessing fish use of these areas utilized *GoPro* video cameras deployed on the bottom, pointing towards the surface (Figure 25). Video sampling occurred in May, July, and September. The video analysis was much more effective at capturing other species than the minnow traps. Some of these fish (Atlantic menhaden & juvenile bluefish) were too large to be captured by a minnow trap. Species observed included:

- silverside, *Menidia menidia*
- mummichog, *Fundulus heteroclitus*
- adult Atlantic menhaden, *Brevoortia tyrannus*
- juvenile bluefish, *Pomatomus saltatrix*
- striped killifish, *Fundulus majalis*
- striped bass, *Morone saxatilis* (formerly *Roccus saxatilis*)
- puffer; likely northern puffer, *Sphoeroides maculatus*
- summer flounder (fluke), *Paralichthys dentatus*

Figure 25: Image from GoPro Camera 3, Deployed at Milford Point South, 9/18/19
The camera is on the bottom, pointing towards the surface. Note the large school of juvenile bluefish. Two striped killifish are captured in this image, one in the foreground on the right and one under the diagonal stem on left.

Fish abundance was low during the May deployment at both sites (Figure 26). July was dominated by silversides at both sites, with very few animals of other species observed. The September observations from Milford Point were dominated by bluefish and silversides and exhibited relatively high time-on-screen for mummichogs and "other", which in this case, were striped killifish. In some cases, large schools of fish pass quickly through the area. In other cases, a smaller schools of fish will remain on screen for the entire video; this tended to be the silversides. The striped killifish in the September Milford Point video were a group of three to five fish that stayed near the camera, and the occasional striped killifish that transited through the field of view.

The September Stratford Point observations are misleadingly low. The cameras were deployed the day after the Milford Point September sampling event, but a storm had moved into the area resulting in large waves and very turbid water. While fish close to the camera were still visible, fish that were more distant could not be observed (Figure 27). In addition, the water movement likely kept fish away from shallow areas, seeking refuge in deeper areas not impacted by the wave action. For the September Stratford Point sampling, the cameras were not placed in the reef balls, but in the marsh grass, similar to the type of environment sampled at the Milford Point site. As a pilot project, we were trying to determine if moving the comparison to the marsh grass provided a better comparison between the sites. Unfortunately, the adverse conditions led to inconclusive results.

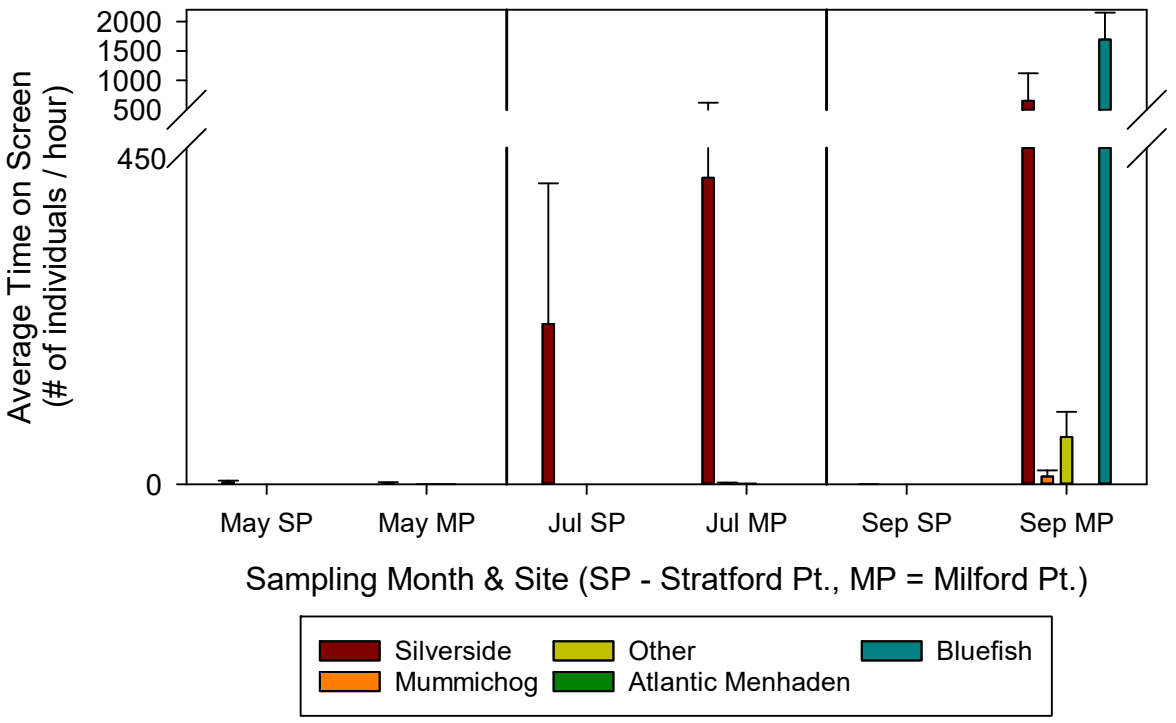

Figure 26: Average Time on Screen for Fish
The average time on screen was calculated by noting when fish appeared on screen and when they left. Bars are the average of four cameras and the standard error.

	Camera 1	Camera 2	Camera 3	Camera 4
5/15 M	 silversides	 silversides	 gull	 silversides
5/30 S	 silversides	 snails	 grass shrimp	 silversides
5/31 M	 silversides	 grass shrimp	 menhaden	 menhaden
7/12 S	 silversides & snails	no data	 silversides	 silversides
7/13 M	 silversides	 mummichog	 silversides	 summer flounder (fluke)
9/8 M	 mummichog	 bluefish & striped killifish	 bluefish & striped killifish	 bluefish
9/9 S	 silverside	 silverside	no data	 silverside
5/29 S	no data	 nothing	 snail	no data

Figure 27: Examples of the Field-of-View for Video Sampling
Each image shows a screenshot from the video by station and by date. Animals visible in the frame are noted under each photo. Note the turbidity on 9/9/18 in Stratford.

The video camera observations were used to evaluate the diversity of the two sites relative to each other. Marshes, in general, have a low diversity compared to other habitats due to the extremes of temperature and availability of water experienced throughout the course of a day as a result of the tides (Keddy 2000). The fish utilizing the marsh tend to be juveniles to small fish, seeking refuge and a place to forage. The September deployment at Stratford Point was not included in the analysis because only ten fish were observed across the three cameras deployed. The species abundance (number of individuals per species) was lower in Stratford Point relative to Milford Point across all sample dates (Table 4). This same pattern was also true for species richness (number of taxa). All three diversity indices (sequential comparison diversity index, Shannon's Diversity index, Simpson's Inverse Dominance index) showed the same patterns, with Stratford Point being consistently and considerably lower than Milford Point.

Table 4: Diversity Indices Based on Video Analysis
Results of the diversity indices are color coded based on relative values; red indicates low diversity; green indicates high diversity and yellow indicates a mid-level diversity. The September data from Stratford Point did not include enough fish observed to be included in the diversity calculations.

	Total Number of Fish Observed	Species Richness (# of taxa)	Sequential Comparison Diversity Index higher = diverse (0 to 20)	Shannon's Diversity Index, H' higher = diverse (0 to 5)	Simpson's Inverse Dominance Index, 1/λ higher = diverse (1 to ∞)
May SP	94	2	0.02	0.00	1.00
Jul SP	174	1	0.02	0.00	1.00
Sep SP	10	2			
May MP	135	5	0.30	0.36	1.29
Jul MP	713	8	0.28	0.25	1.14
Sep MP	3462	6	0.28	0.44	1.27

The species richness observed by minnow traps versus video observations reveals that different species are captured by the two methods (Table 5). As mentioned previously, Atlantic menhaden and bluefish are not sampled by the minnow traps as these individuals are too large for the trap. The video at Stratford Point was from within the reef balls and likely missed the mummichogs and striped killifish captured by the traps deployed outside the reef balls because these species do not hover over the reef balls as silversides do, tending to spend more time near the bottom.

Table 5: Observed Fish Species
The check marks indicate the species was observed at the site on video or in the minnow traps.

species	Milford Point		Stratford Point	
	video	trap	video	trap
silverside, *Menidia menidia*	✓	✓	✓	✓
mummichog, *Fundulus heteroclitus*	✓	✓		✓
striped killifish, *Fundulus majalis*	✓	✓		✓
adult Atlantic menhaden, *Brevoortia tyrannus*	✓			
juvenile striped bass, *Morone saxatilis*		✓		✓
adult striped bass, *Morone saxatilis*	✓			
juvenile bluefish, *Pomatomus saltatrix*	✓		✓	
puffer, likely northern puffer, *Sphoeroides maculatus*	✓			
summer flounder (fluke), *Paralichthys dentatus*	✓			

5 Discussion

Several initiatives have advocated for more studies of the blue carbon[3] potential of marshes and other wetland habitats, and attempted to determine a path towards commercializing carbon sequestration and storage as a commodity (Howard et al. 2017; Rodosta et al. 2011). Within the last five years, the Intergovernmental Panel on Climate Change (IPCCC) has developed guidelines for assessing coastal blue carbon stores and have included them in their carbon accounting (IPCC 2014). As society becomes more aware of the importance of reducing our collective carbon footprint, accurately assessing and managing blue carbon stores is going to become increasingly important. Restored marshes have the potential to contribute to carbon storage, but in urbanized estuaries like Long Island Sound where space is limited and many of our marshes are previously disturbed, the question becomes, "do these marshes deliver the same benefits and how long does it take to achieve parity with natural, mature marshes?"

5.1 Carbon Sequestration

The carbon sequestration varies among the sites sampled, with the established natural fringing marshes having average carbon sequestration rates ranging from 88 ± 18 g m^{-2} y^{-1} and 180 ± 5 g m^{-2} y^{-1} (average and standard error). The marsh meadow platform at Charles Wheeler North fell within the range seen at the established fringing marshes, with an average carbon sequestration rate of 112 ± 13 g m^{-2} y^{-1}. The restored marshes (4-year old SN and 1-year old SS) showed lower carbon sequestration rates, with the older restored marsh having a greater carbon sequestration rate than the younger (27 ± 6 g m^{-2} y^{-1} vs. 4 ± 1 g m^{-2} y^{-1}). As these restored marshes age, plant biomass and coverage will increase and should further help to accumulate sediment and carbon in these sites. Recovery time for marshes ranges between five and twenty-five years (Borja et al. 2010; Craft et al. 2003), thus these newly restored marshes are just beginning the journey towards maturity.

Returning to the same stations at four times over the year (April, June, August, October) revealed that sampling between mid-April and mid-August will yield carbon sequestration rates consistent throughout the year and with other studies. Sampling in October, when the below-ground biomass is at its maximum carbon content, will overestimate the carbon sequestration rate.

The sediment quality in the fringing marshes (MN, MS, SN, SS) tended to be rocky and harbor a diverse assemblage of organisms, including mussels, oysters, slipper snails, and fiddler crabs. These organisms bioturbate the sediment, mixing the surface sediment. In addition, the Milford Point South site and both restored sites at Stratford Point are subject to wave action. These conditions mean that sediment marker horizons are not good options for estimating sediment accretion. LIDAR data from Stratford Point highlighted areas that were accreting sediment and areas experiencing scour, but the error on these estimates are high (Mattei 2018). Future work will include the installation of sediment plates at each station to better estimate short-term sediment accretion rates.

While this study provided a comparison of restored marshes to natural mature marshes in a nearby area, putting these rates into a global context requires a summary of values found in the primary literature. Carbon sequestration rates (g m^{-2} y^{-1}) for salt marshes around the globe were gathered,

[3] Blue carbon is carbon fixed by coastal ocean ecosystems, rather than terrestrial systems.

resulting in 120 separate estimates. Carbon bulk density (g cm^{-3}) was also compared using 130 separate estimates from the around the globe. These data were divided by region of the ocean and country, with the long expanse of the United States' and Canada's coastlines broken into regions. Each station estimate from this study was included in the comparison, with this study's estimates set apart from the literature values for comparison. This study yielded 43 estimates of carbon sequestration and carbon bulk density for natural marshes and 20 for restored marshes (Figure 28).

A Kruskal-Wallis one-way ANOVA on ranks on the carbon bulk density indicated there was a statistically significant difference among the regions ($\chi^2(14) = 84.706$, $p < 0.001$). The Dunn's Method Pairwise Comparison indicated the Atlantic Region of Canada, the UK, the Northeast USA, and the Gulf of Mexico were statistically significantly different from the marshes in this study (restored and natural) and the Southeast USA; all other pairwise comparisons were not statistically significantly different (Figure 28).

A Kruskal-Wallis one-way ANOVA on ranks on the carbon sequestration rate indicated there was a statistically significant difference among the regions ($\chi^2(10) = 56.377$, $p < 0.001$). The Dunn's Method Pairwise Comparison indicated the Atlantic Region of Canada, the Netherlands, Mid-Atlantic USA, and Gulf of Mexico were statistically significantly different from the restored marshes in this study and the Southeast USA; all other pairwise comparisons were not statistically significantly different (Figure 28).

In general, the comparison with literature values confirms the conclusion that the restored marshes evaluated in this study have lower carbon sequestration rates but are already surpassing marshes from some areas of the world. Future work in these restored marshes will better estimate the sediment accretion rate and more importantly, will track these marshes as they mature and develop into habitats equivalent to natural fringing marshes, providing a better understanding of the path to recovery.

5.2 Fish Use of Reef Balls

Fish use of the reef balls was evaluated using two methods: baited minnow traps and video observations. The video cameras were placed in the reef balls, facing the surface (Figure 27). In comparison, the video cameras at the reference marsh at Milford Point were placed at the seaward edge of the marsh plants, also facing the surface. The minnow traps were deployed outside the reef balls, often near marsh plants, similar to the position of minnow traps at Milford Point.

Both sampling methods had their biases. The video cameras at Stratford Point did not capture the mummichogs and striped killifish that were in the area because these fish tend to stay near the bottom and do not utilize the interior or top of the reef ball, though they may be around the outside base of the reef balls. Positioning the video cameras outside the reef balls would be a more appropriate comparison to the deployment at Milford Point and will be used moving forward as we continue to monitor these sites. The minnow traps, while deployed in a similar setting at each site, exclude larger fish.

Even with these sampling biases, we can see that biomass of fish increases through the year, with the peak biomass observed during our mid-August sampling (Figure 24). More traps would need to be deployed to adequately compare sites (at least 112 traps per sampling event), making the use of minnow traps not ideal for our purposes. These initial results hint at the fact that we are likely to find greater biomass at the Milford Point North site, a shallow enclosed tidal flat, while the more exposed area at Milford Point South is likely to be similar to the Stratford Point biomass.

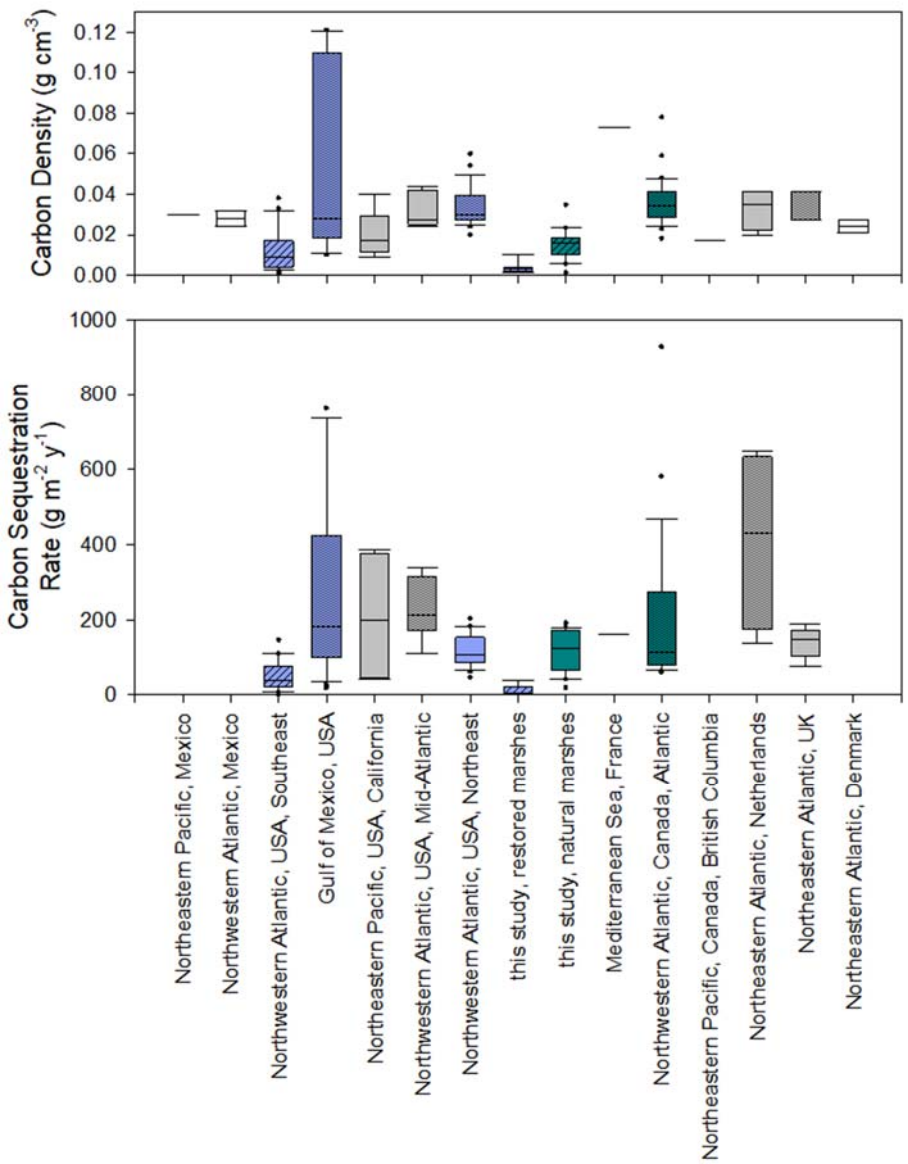

Figure 28: Literature Values for Carbon Sequestration and Carbon Bulk Density
Literature estimates for carbon sequestration and carbon bulk density were divided by region. Data from this study were divided into the natural marshes and the restored marshes. Stippling indicates these areas are statistically significantly different from the striped bars; all other comparisons showed no statistically significant differences. The colors of the bars indicate the number of estimates included for each region: white = 1 – 2, grey = 5 – 7, light blue = 20 – 26, cyan = 34 – 42. Data were gathered from: (Adame et al. 2013; Adame et al. 2015; Artigas et al. 2015; Craft et al. 2003; Drake et al. 2015; Howes et al. 1985; Nahlik and Fennessy 2016; Radabaugh et al. 2018) and from sources cited in Chmura et al. (2003), including: (Bryant and Chabreck 1998; Cahoon 1994; Cahoon et al. 1996; Cahoon and Turner 1989; Callaway and DeLaune 1997; Callaway et al. 1996; Chmura and Hung 2004; Connor et al. 2001; Craft et al. 1993; French and Spencer 1993; Hensel et al. 1999; Kearney and Stevenson 1991; Markewich et al. 1998; McCaffrey and Thomson 1980; Morris and Jensen 1998; Oenema and DeLaune 1988; Orson et al. 1998; Patrick and DeLaune 1990; Roman et al. 1997).

The video sampling proved to be more robust than the trap sampling, providing an adequate sample size to compare among sites and sample dates. Both the species richness (number of taxa) and species diversity were greater at the Milford Point site relative to Stratford Point. However, this comparison was between the marsh grasses at Milford Point and the reef balls at Stratford Point. Future work will compare the marsh grasses at Stratford Point to Milford Point's marsh grasses. We can conclude from this work that the diversity of species utilizing the reef balls is considerably lower than those using marsh grasses, though we would also expect to find this pattern when comparing the marsh grasses at Stratford Point to the reef balls at Stratford Point.

6 Conclusion

The restored fringing marshes at Stratford Point are on their way towards achieving similar levels of carbon sequestration as natural fringing marshes, but as expected by their age (four-years-old & one-year-old), are still falling short. Recovery time for marshes ranges between five and twenty-five years (Borja et al. 2010; Craft et al. 2003), thus these newly restored marshes are just beginning the journey towards maturity. The dynamic nature of the sediment at the Stratford Point site, a result of wind and wave action, may make this site slower to achieve full parity with natural fringing marshes. The Milford Point South site provides a good reference for Stratford Point as it has a similar rocky soil type and exposure to wind and wave action.

In the future, fish sampling protocols should be revised to better compare the Stratford Point marsh to the Milford Point marsh. The video sampling was successful at capturing the community composition and abundance of species and shows great promise as a metric for comparing fish use of these areas.

The marsh grasses are expanding through the intertidal area of Stratford Point, suggesting that the restoration efforts are positively impacting the return of a living shoreline to the formerly heavily impacted Stratford Point coastal area. Continued monitoring of Stratford Point will chart its' progress towards developing into a mature and fully functional fringing marsh, providing insight into the trajectory of recovery for living shorelines.

Stratford Point, fall 2018. Photo credit: Jamie Vaudrey (CC BY-NC-SA 4.0)

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8 Appendix A – Sediment Analysis SOP

POINT OF CONTACT
NAME: Jamie Vaudrey
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I. OBJECTIVE: Analysis of sediment organic content and bulk density of marsh sediment cores.

II. OVERVIEW: Three or more marsh sediment cores were collected in the field from a station, composited, and divided into sections based on depth of the sediment. Ideally, each depth sample was separated into three bags: one bag for analysis of the whole sample, one bag for analysis of plant material, and a small subset of the other two samples for determining water content. If this separation was not done in the field, it must be done in the lab prior to analysis. In the lab, samples are processed to determine bulk density, organic content is determined via loss on ignition (LOI), and carbon content is determined on a subset of samples via elemental analysis.

III. SCOPE AND APPLICABILITY: Review paper by Heiri et al. (2001) and Santisteban et al. (2004).

IV. SAFETY, CAUTIONS, AND LIMITATIONS:

V. MATERIALS AND EQUIPMENT:

Field Sampling

Large Whirl-paks, Fisherbrand™ Sterile Sampling Bags with Flat-Wire Closures (FisherSci 14-955-190, $181.59 / case of 2 pack, where a pack has 500 bags)

Avery Matte Frosted Clear Return Address Labels for Laser Printers, 1/2" x 1-3/4", 2,000 Labels (5667 or 15667 for 800 labels)

Russian Peat Borer, borrow from teaching lab (Wildco Flag Sampler - Russian Peat Borer, Part # 3-2460- F20; $1,327 + $125 shipping)

knives

cutting surface

rebar stakes and protector caps

GPS unit

Equipment

- freezer
- refrigerator

Incubator capable of holding temperature at 50°C (Vaudrey Lab, Thelco Precision Laboratory Incubator, tall oven with the red handle)

Oven for 105°C (Vaudrey Lab, Thermo Scientific™ Precision™ Compact Oven)

Muffle Furnace (SMALER Lab, Fisher Scientific Isotemp Programmable Muffle Furnace, use the one on the

Bel-Art Science Micro-Mill grinder with hard-faced blade (Vaudrey lab, tall blue grinder)

Ohaus Analytical Precision Plus Balance (Vaudrey Lab)

Ohaus Precision Top Loading Balance (Vaudrey Lab)

Sediment Samples

- data sheets
- porcelain mortar and pestle

- scissors
- rubber stoppers
- plastic tray
- round-tip paint brushes
- crucible tray
- crucible tongs
- porcelain crucibles, high form, 30 mL, max. temp. of 1150°C (FisherSci FB965E, $33.18 / 6)
- porcelain crucible covers, 53mm diameter cover (FisherSci FB965U, $40.34 / pack of 6)
- large aluminum roasting pans, disposable (purchase at Job Lot)
- nitrile gloves (FisherSci; small 19-130-1597B; medium 19-130-1597C; large 19-130-1597D; $5.14 / pack of 100)
- Kimwipes, 8.4 x 4.4 in. (FisherSci 06-666A, $2.29 / pack of 280)
- aluminum foil (purchased at BJs)

Bulk Density Correction and Elemental Analysis

- data sheets
- porcelain mortar and pestle
- scissors
- aluminum cups, Fisherbrand™ Aluminum Weighing Dishes, 65mL with fluted sides (FisherSci 08-732-102, $22.00 / pack of 100)
- ballpoint pen & rubber stopper
- plastic tray
- round-tip paint brushes
- scintillation vials, Fisherbrand™ 20mL Borosilicate Glass Scintillation Vials, White Polyethylene Caps, no liner (FisherSci 03-337-14, $138.67 / case of 500)
- cardboard tray for holding scintillation vials
- Avery Matte Frosted Clear Return Address Labels for Laser Printers, 1/2" x 1-3/4", 2,000 Labels (5667 or 15667 for 800 labels)
- nitrile gloves (FisherSci; small 19-130-1597B; medium 19-130-1597C; large 19-130-1597D; $5.14 / pack of 100)
- Kimwipes, 8.4 x 4.4 in. (FisherSci 06-666A, $2.29 / pack of 280)
- aluminum foil (purchased at BJs)
- ASTM Type I water (from SMALER lab), for rinsing scintillation vials

Plant Samples

- datasheets
- *aluminum pie plates* - Fisherbrand™ Disposable Aluminum Dishes with Fluted Sides, 180mm diameter, 500 mL (FisherSci 08-732-109, $10.27 / pack of 50)

- ballpoint pen & rubber stopper
- scissors
- 500 micrometer mesh sieve, 21 cm diameter
- plastic rinse bottle
- plastic tray
- nitrile gloves (FisherSci; small 19-130-1597B; medium 19-130-1597C; large 19-130-1597D; $5.14 / pack of 100)
- paper towels
- tap water

VI. METHODS

Preparation, prior to field collection date.

1. Ask Jamie or Jo-Marie to print labels and datasheets – this is to avoid hand-writing the labels on bags and datasheets.
2. Remove plastic sealing tabs from Whirl-paks.
3. Apply labels to Whirl-paks.
4. Weigh each sample bag on the Ohaus top loading balance (***without*** the glass doors), record the weight of the bag on the bag with a sharpie (in white area on bag) and record on the appropriate data sheet.

Preparation, prior to analysis.

1. Rinse 20 mL glass scintillation vials with ASTM Type I water (from the SMALER lab) and allow to dry. You will need one vial for each "B" sample.
2. Ask Jamie or Jo-Marie to print labels for the scintillation vials, for the "B" samples.
3. Clean crucibles if they have not been used within the last 6 weeks ***or*** if they have been sitting exposed and not covered in aluminum foil.
    a. remove all sediment with a brush or by rinsing and allowing to dry completely
    b. muffle the crucible for 4.5 hours at 550°C
    c. store crucibles upside down on a metal tray lined with fresh aluminum foil and cover crucibles with fresh foil
    d. crucible covers should be stored wrapped in aluminum foil so they are not exposed on any side, so they are wrapped in a foil packet (as a group, not individually)

Sample Storage, upon return from field.

- Weigh each bag and sample on the Ohaus top loading balance (***without*** the glass doors) to get the wet weight and record on the datasheet.
- Place bags with an "S" and those with a "B" at the end of the label into the 50°C incubator oven (oven with red handle). Be sure to open each bag, making the mouth opening wide, and place all bags into a large aluminum pan (do not just set the bags in the oven).

- Place the bags with a "P" at the end of the label into the sediment freezer located in the lab or in the walk-in freezer on the second floor. If you will start processing these samples the next day, they may be stored overnight in the black refrigerator. Do not store sediment or plants in a refrigerator or freezer designated for nutrients (do NOT store these samples in the white refrigerator in the lab or the tall white freezer in the lab anteroom).

Processing Sediment ("S") Samples

Work with only one sample at a time.

1. Dry completely in the incubator (oven with red handle) at 50°C (approximately 48 - 96 hours).
2. Close the bag by folding the bag over a few times and folding in the metal tabs. DO NOT place an open bag on the balance or on the counter unsupported as material may spill out.
3. Weigh dry sample in bag on the Ohaus top loading balance (***without*** the glass doors) and record weight of bag and sample on datasheet.
4. RETURN TO INCUBATOR – Open bag and return to incubator for an additional 24 hours.
    a. Repeat steps 2 & 3.
    b. If weight is lower, then sample was not dry on previous day. Return to oven for another 24 hours.
    c. Repeat this process until weight does not change over a 24-hour period – ask Jamie for what is an acceptable change between days.
5. Work on a clean tray so that you can recover any sample that is spilled while transferring sample from the bag to the Micro-Mill.
6. If there are any large chunks of sediment (>2cm), use a mortar and pestle to break large chunks and grind down large pieces of plant matter. You may use scissors to cut root material into smaller pieces.
7. Return ground sample back to the original Whirl-pak, if you are not proceeding with the following steps immediately.
8. Determine the number of crucibles needed by dividing the weight of the sample by 3 g.
9. Weigh empty crucibles on the OHAUS Analytical balance (tall balance with glass doors) and record crucible identification numbers and weight on datasheet. *REMOVE THE LID WHILE WEIGHING*.
10. Portion entire sample into approximately 3 g portions into each crucible.
11. Record weight of crucibles with sediment samples, using the Ohaus Analytical balance (tall balance with glass doors).
12. Put crucibles with sample on crucible rack ~1 in apart and add lids to crucibles. If lids are not available, cover the whole tray with foil to keep dust from settling in the samples.
13. Turn on the Thermo Scientific™ Precision™ Compact Oven located on the counter across from the computer desk and allow the oven to come to 105°C ***before*** you put anything in the oven. Use the thermometer on the top of this oven to confirm that the oven is at the correct temperature before you put samples in the oven.

    Recall, this oven is not like your kitchen oven – it will not maintain a set temperature but instead puts out a set amount of energy over time; if you put liquid in the oven, the temperature will be lower until the liquid has all boiled off, then the oven will return to 105°C.

Thus, if you turn the temperature up because it looks like it is reading low and you go home for the night, as the water boils off, the temperature will get very high. This is why you must confirm the temperature is correct BEFORE you put anything in the oven and then you must leave the dial alone, regardless of what the oven reads while the samples are drying

14. Around 3 p.m., put crucibles into the 105°C oven and allow to heat overnight (18 to 24 hours).
15. Allow samples to cool. Be sure to cover with foil to keep dust from settling on the crucibles if they do not have lids.
16. Weigh crucibles with sample and record weight on the datasheet. *REMOVE THE LID WHILE WEIGHING*.
17. Place crucibles in muffle oven at 550°C for 4.5 hours. This is the total time, from start to finish, and includes about 25 minutes of preheating. Thus, the burn at 550°C is about 4 hours.
18. Allow samples to cool. Be sure to cover with foil to keep dust from settling on the crucibles.
19. Weigh crucibles with sample and record weight on the datasheet. *REMOVE THE LID WHILE WEIGHING*.
20. Return crucibles with sample to 550°C oven for 1 hour. This includes the total time, including the pre-heating time.
21. Re-weigh samples and record weight. *REMOVE THE LID WHILE WEIGHING*. If weight has changed by more than 5% (of organic matter), return to oven for 1 hour and repeat heating and weighing until weight is unchanged.

Processing Bulk Density Correction and Elemental Analysis ("B") Samples

Work with only one sample at a time.

1. Dry completely in the incubator (oven with red handle) at 50°C (approximately 48 - 96 hours).
2. Close the bag by folding the bag over a few times and folding in the metal tabs. DO NOT place an open bag on the balance or on the counter unsupported as material may spill out.
3. Remove sample from 50°C incubator and weigh dry sample in bag on top-loading balance (the one ***without*** the glass doors) and record weight of bag and sample on datasheet.

   NOTE – keep this sample in the incubator for the same amount of time as the "S" samples. By doing this, you only need to weigh the "S" samples for a few consecutive days to test for dryness, not these. You can assume these are dry when the "S" samples are dry.
4. Save this sample's labelled Whirl-pak, you will need it later.
5. Work on a clean tray so that you can recover any sample that is spilled while transferring sample from the bag to the Micro-Mill.
6. If there are any large chunks of sediment (>2cm), use a mortar and pestle to break large chunks and grind down large pieces of plant matter. You may use scissors to cut root material into smaller pieces.
7. You will eventually grind the whole sample down to sizes of < 2 mm.
   a. For tough samples, use the Micro-Mill, but you may need to work in subsamples, depending on the size of the sample. Place sample into the Micro-Mill and grind for 30 seconds in intervals of

10 seconds, being sure that the Micro-Mill does not overheat. You may need to hook the Micro-Mill to a cooling bath (ask Jamie) if you are working with many samples in a row.

b. The coffee grinders are good for plant samples.

8. Find the label for the sample you are working with and place on a 20 mL glass scintillation vial that has been rinsed with ASTM type I water (from SMALER lab) and thoroughly dried.
9. Prepare a small aluminum cup for heating the subsample for elemental analysis.
   a. *Note* – the ID on these cups should be abbreviated. A typical ID looks like:
      "**1806**-CN-**A**/corer **00**-10 B", which can be abbreviated as: "1806 A 00".

   b. Inscribe the sample ID and weight on bottom of aluminum cup using a ball point pen; place the cup on a rubber stopper and press down as you write to make an indentation in the metal surface. Be sure that you have flipped the cup over – you should be writing on the bottom of the cup, not the inside of the cup. *We use this inscribing technique because sharpies can wear off when working with sediment.*
10. Fill the scintillation vial ¾ full with sample. Pour the sample into the prepared aluminum cup.
11. Locate the Whirl-pak that corresponds to this sample. Double-check you have the correct Whirl-pak and return the remainder of the sample to the Whirl-pak. Store the sample in the designated cardboard banker's box, in the correct folder or bag for the sample date.
12. Turn on the Thermo Scientific™ Precision™ Compact Oven located on the counter across from the computer desk and allow the oven to come to 105°C ***before*** you put anything in the oven. Use the thermometer on the top of this oven to confirm that the oven is at the correct temperature before you put samples in the oven.

    Recall, this oven is not like your kitchen oven – it will not maintain a set temperature but instead puts out a set amount of energy over time; if you put liquid in the oven, the temperature will be lower until the liquid has all boiled off, then the oven will return to 105°C. Thus, if you turn the temperature up because it looks like it is reading low and you go home for the night, as the water boils off, the temperature will get very high. This is why you must confirm the temperature is correct BEFORE you put anything in the oven and then you must leave the dial alone, regardless of what the oven reads while the samples are drying

13. Around 3 p.m., put aluminum cup into one of the three the aluminum baking trays designated for use in the Thermo Scientific™ Precision™ Compact Oven.
14. Cook sample overnight at 105°C, for 18 to 24 hours.
15. Transfer the sample to the glass scintillation vial. Place in a cardboard tray and store on the shelf by the computer desk for later analysis.

Processing Plant ("P") Samples

Work with only one sample at a time.

Note – you may start this analysis when the core samples are frozen. If frozen, you will need to let the core sit for longer in the water to allow the core to thaw.

1. Prepare an aluminum pie plate for determination of plant fraction.

a. Weigh aluminum pie plate on the Ohaus top loading balance (***without*** the glass doors) and record weight on data sheet.
b. *Note* – the ID on these plates should be abbreviated. A typical ID looks like:
"**1806**-CN-**A**/corer **00**-10 P", which can be abbreviated as: "1806 A 00".
c. Inscribe the sample ID and weight on bottom of aluminum pie plate using a ball point pen; place the cup on a rubber stopper and press down as you write to make an indentation in the metal surface. Be sure that you have flipped the pie plate over – you should be writing on the bottom of the pie plate, not the inside of the pie plate. *We use this inscribing technique because sharpies can wear off when working with sediment.*

2. Work on a clean tray so that you can recover any sample that is spilled.
3. Place the wet core sample in the labelled aluminum pie plate. Rinse sample bag and empty rinse water into aluminum pie plate. Let soak for at least 1 hour to allow sediment to settle.
At this stage, sample may be put in refrigerator overnight for storage. If this is necessary, cover the pan tightly with aluminum foil. Be sure that sample will not spill in the refrigerator.
4. Prepare a wash bottle with tap water and a wash basin for rinsing the sample. DO NOT rinse the sample into the sink; rinse water cannot go down the drain, the sediment will clog the drain.
5. Position the sieve (21 cm diameter, 500 µm mesh size) over the wash basin.
6. Pour contents of the aluminum pie plate into the sieve. Rinse the pie plate clean, directing rinse water into the sieve.
7. Using large, blunt-tip tweezers or gloved hands, spread the plant matter out into a thin layer on the sieve.
8. Over the wash basin, rinse plant matter repeatedly until rinse water runs clear.
9. Place cleaned plant matter back into the labelled aluminum pie plate.
10. Dry sample in the incubator at 50°C for at least 48 hours and until completely dry.
11. Pour rinse water into a 5-gallon bucket and allow sediment to settle overnight or when the water is clear. Decant off liquid and allow remaining liquid to evaporate, then dump sediment into the trash. You may not pour sediment down the drain nor into the estuary. Additional buckets are available in the cage in the basement.
12. Weigh aluminum pie plate and dried plant sample on the top loading balance (***without*** the glass doors) and record the weight.
13. RETURN TO INCUBATOR – for an additional 24 hours.
a. Repeat steps 10 & 12.
b. If weight is lower, then sample was not dry on previous day. Return to oven for another 24 hours.
c. Repeat this process until weight does not change over a 24-hour period – ask Jamie for what is an acceptable change between days.
14. Write the sample ID label near one end of a long piece of foil (~54 cm long) using a sharpie. Use the piece of aluminum foil to completely encircle the pie plate, sealing in the sample. The sample ID should be on the top of the sample, clearly visible. Leave enough slack in the foil that plates can be

stacked closely on top of one another without ripping the foil. Store in the labelled plastic bin in the walk-in freezer on the second floor.

VII. Data Analysis

Enter data into template, as instructed by lab manager. Check data at a later date and record in the Excel file that data were checked.

VIII. Works Cited

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9 Appendix B – Sediment Station Locations

Site Code	Station ID Letter	Latitude (dec. deg.)	Longitude (dec. deg.)	coring method
CN	A	41.19305	-73.10117	Russian peat corer
CN	B	41.19286	-73.10136	Russian peat corer
CN	C	41.19345	-73.10133	Russian peat corer
CN	D	41.19317	-73.10121	Russian peat corer
CN	E	41.19322	-73.10065	Russian peat corer
CN	F	41.19368	-73.10034	Russian peat corer
SS	G	41.15515	-73.10390	digging
SS	H	41.15521	-73.10369	digging
SS	I	41.15505	-73.10422	digging
SS	J	41.15503	-73.10455	digging
SS	K	41.15490	-73.10481	digging
SS	L	41.15506	-73.10506	digging
SN	M	41.15504	-73.10540	digging
SN	N	41.15495	-73.10555	digging
SN	O	41.15506	-73.10566	digging
SN	P	41.15511	-73.10591	digging
CS	Q	41.17643	-73.10165	Russian peat corer
CS	R	41.17660	-73.10156	Russian peat corer
CS	S	41.17694	-73.10108	Russian peat corer
CS	T	41.17714	-73.10110	Russian peat corer
MN	U	41.17476	-73.10034	digging
MN	V	41.17447	-73.10062	digging
MN	W	41.17429	-73.10062	digging
MN	X	41.17402	-73.10090	digging
MS	Y	41.17274	-73.10538	digging
MS	Z	41.17269	-73.10570	digging
MS	AA	41.17243	-73.10577	digging
MS	BB	41.17237	-73.10611	digging
MS	CC	41.17234	-73.10651	digging
MS	DD	41.17227	-73.10682	digging