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Results of 2013 Macroalgal Monitoring and Recommendations for Future Monitoring in Great Bay Estuary, New Hampshire

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Abstract

The recently designated nitrogen impairment and reports of elevated macroalgal growth in Great Bay Estuary indicate ecological imbalance. However, reversing the Estuary's ecological decline will require commitment of considerable resources and is complicated by the variety of sources that deliver nitrogen to the Estuary and the intermittent nature of historic macroalgal monitoring. To advance our understanding of the macroalgal and nitrogen dynamics of the Estuary, data were collected via three approaches: 1) assessing plant cover and biomass along transects; 2) assessing plant cover at randomly selected points; and 3) comparing the nitrogen isotope ratios of macroalgae collected from different habitats. The results offer insight into changes in macroalgal abundance and species composition and the relative importance of various nitrogen sources to macroalgae in Great Bay. Overall, our results corroborate the findings of increasing macroalgal blooms in previous studies and suggests plausible directions for a long-term macroalgal monitoring program.

Introduction

Because Great Bay Estuary is recognized as an estuary of national significance under U.S. EPA's National Estuary Program (NEP) and is one of the 28 estuaries included in NOAA's National Estuarine Research Reserve System (NERRS), it is in a position to be a leader in estuarine sampling and monitoring. As one of the estuaries in the NEP and the NERRS, it is also of national interest to protect the ecological integrity of Great Bay Estuary and invest in understanding how its ecological processes vary through space and time and are impacted by human activities in the watershed. In 2010, researchers observed that the abundance of macroalgae had increased dramatically since the previous study (Nettleton et al., 2011), conducted thirty years earlier (Hardwick-Witman and Mathieson, 1983). Such a dramatic change led the PREP to identify a long-term macroalgal monitoring program as a monitoring priority for Great Bay Estuary in its 2013 State of Our Estuaries Report (PREP, 2013).

Tracking changes in macroalgal populations is a fundamental piece of information required for understanding how changes in the environmental conditions of Great Bay Estuary, particularly nutrient loads and cycling, affect biodiversity in the Estuary. Mats of macroalgae can intercept the sunlight needed for eelgrass (*Zostera marina* L.) growth (Beem and Short, 2009), altering the habitat structure and food web of the estuary (Deegan et al., 1997). Nitrogen fertilization can increase macroalgal growth, producing blooms (Bricker et al., 2008) due to nitrogen's role as the limiting nutrient in coastal systems (Herbert, 1999). Eleven of the eighteen assessment zones in Great Bay Estuary were listed as impaired due to excess nitrogen under Section 303(d) of the Clean Water Act in 2008 (NHDES, 2012). Increases in nitrogen loading to Great Bay Estuary may reflect increasing human populations and sewer discharge. The fact that eighteen local communities rely on Great Bay Estuary to assimilate discharges from their wastewater treatment facilities complicates the process of developing a nitrogen management plan for the Estuary; the cost of capitalization and operation of these facilities will increase if they are required to reduce the nitrogen loads they deliver to the Estuary (Sanborn, 2011). Because of the role that runoff plays in nitrogen loading to Great Bay, changes in nitrogen concentrations in the Estuary may also reflect land use changes upstream in the watershed or changes in precipitation patterns due to climate change.

We undertook this study in an effort to quantify the abundance of macroalgae in Great Bay Estuary and to describe the dominant species composition and distribution in the Estuary. Based on previous research in the Estuary, we developed a three-part research approach. In the first approach, percent cover and biomass data were collected by species/genus on permanent transects at four intertidal sites at four different times of year. The second approach tested the possibility of using random sampling to collect data that were more representative of the entire Estuary over a 1-month time period. In the third research approach, isotope tracing was used to investigate the contributions of different nitrogen sources to macroalgae collected from 20 sites dispersed throughout the Estuary.

Transect-based vegetation sampling has become increasingly common in estuaries in the NEP and NERRS networks (Rumrill and Sowers, 2008) in the last ten years. In Great Bay Estuary specifically, two past studies have collected macroalgal cover data via transect sampling. Hardwick-Witman and Mathieson (1983) used transect sampling at four sites to investigate spatial and temporal gradients in the species composition of intertidal biota, including

macroalgae. Transect sampling was not formally used to study macroalgae in Great Bay Estuary again until thirty years later. With the objective of investigating the environmental status of the Great Bay National Estuarine Research Reserve, Nettleton et al. (2011) collected abundance and distribution data for macrophytes from five sites, two of which were loosely based on Hardwick-Witman and Mathieson's sites, between September of 2008 and July of 2010.

Although Hardwick-Witman and Mathieson's and Nettleton's studies had different purposes, the percent cover data from both studies are comparable because the authors used the same data collection methods. It is uncertain what the trend may have been in the intervening years, but Nettleton observed a significant increase in the cover of the green algal genus *Ulva* at the Lubberland Creek site from < 1% cover observed by Hardwick-Witman and Mathieson in 1979 to an average cover value of 39% in November of 2008. In order to provide comparable data that can be included in future reports, we used four of Nettleton's five sampling sites and modified his methodology to monitor macroalgal abundance and species composition via transect sampling in Great Bay Estuary in 2013. In this way, our first research approach assessed both the current trends in macroalgal species composition and distribution and the suitability of transect sampling for a long-term macroalgal monitoring program.

Previous system-wide habitat studies in Great Bay Estuary have estimated the extent of eelgrass beds (Short, 2009 and 2013) and saltmarsh habitat (Trowbridge, 2006) using aerial photography and remote sensing data in ArcGIS, respectively. The extent of both eelgrass beds and salt marshes appear to have declined in Great Bay Estuary in recent years. Pe'eri et al. (2008) published a map of both eelgrass and macroalgae distribution in Great Bay Estuary using hyperspectral imagery; however, they also identified a lack of groundtruthing data as a weakness in their analysis.

The expectation that aerial photographs would be taken again in 2013 and the knowledge that insufficient groundtruthing data have limited the utility of past remote sensing studies provided the impetus for testing a macroalgal sampling plan that would function like a groundtruthing survey in our second research approach. We tested a random sampling survey in late summer of 2013. The survey provided data that allowed me to summarize the distribution and abundance of macroalgae, eelgrass, and saltmarsh vegetation in Great Bay Estuary during the peak biomass season.

Because of the causal relationship between increases in nutrient availability and algal blooms, several different algae-based indicators of nitrogen loading have been examined in eutrophied estuaries such as Great Bay Estuary. The PREP analyzed the abundance of phytoplankton in Great Bay Estuary as measured by chlorophyll-a concentrations between 1974 and 2011 and was unable to identify a consistent trend (PREP, 2013). Nettleton et al. (2011) were unable to detect significant variation in the nitrogen content of *Ulva* tissue collected from different sites around Great Bay Estuary or *Ulva* tissue collected from the Estuary at different times of the year. A third algae-based indicator of nitrogen loading that had not been tested in Great Bay Estuary prior to this study is the ^{15}N isotope signatures of macroalgal tissue.

The objective of our third research approach was to perform a coarse screening of nitrogen isotope ratios around Great Bay Estuary to see if it would be possible to identify areas where anthropogenic sources contribute significantly to the nitrogen that macroalgae take up. The heavy-weight nitrogen isotope, ^{15}N, is concentrated in metabolic waste produced by humans,

pets, and wildlife due to the fact that molecules containing the lighter-weight isotope, ^{14}N, are preferentially used in processes that are facilitated by enzymes (McClelland and Valiela, 1998; Montoya 2008). In the case of human metabolic waste, the ^{15}N signature is amplified both within the human body as it carries out daily functions and again at wastewater treatment facilities that provide secondary treatment through anaerobic denitrification. Consequently, the discharge of wastewater treatment facilities contributes nitrogen with a high proportion of molecules containing ^{15}N to receiving waterbodies (France, 2011).

Differences in the growth habits of various types of macroalgae make some types of macroalgae better-suited to particular monitoring goals than others. We chose to analyze the ^{15}N signatures of *Ulva* tissue for this study. We felt that this alga would make a good indicator of nitrogen loading in Great Bay Estuary because it can be found in many parts of the Estuary, and its ^{15}N signature reflects recent environmental conditions (2-3 weeks; Aguiar et al., 2003). As is recommended in the literature (Owens, 1987), we used carbon isotope analysis to confirm our interpretation of the ^{15}N analysis, because we did not collect samples representing the ^{15}N signatures of nitrogen sources, such as effluent from wastewater treatment facilities or runoff from fertilized land. As in nitrogen isotope ratios, selective use of ^{12}C increases the proportion of ^{13}C relative to ^{12}C in the waste products of organisms that occupy higher trophic levels. There are also numerous published studies of ^{15}N signatures in *Ulva* that can provide a basis for comparison for our results (Raimon et al., 2013; Titlyanov et al., 2011; Pruell et al., 2006).

Methods

1. Transect Sampling

Site Establishment

Between May and November of 2013, we collected biomass and percent cover data by seaweed taxon at four sites around Great Bay Estuary: Cedar Point in Durham, N.H.; Wagon Hill Farm in Durham, N.H., west of the public beach; The Nature Conservancy's Lubberland Creek Preserve in Newmarket, N.H.; and the Great Bay Discovery Center off of Depot Road in Greenland, N.H. (Figure 1). We chose these sites because Nettleton (2011) collected the same types of data for all four of these locations. In addition, Hardwick-Witman and Mathieson (1983) collected macroalgal biomass and percent cover data at Lubberland Creek, and Chock (1983) collected the same types of data at Cedar Point.

The 2013 macroalgal monitoring team established sampling locations (Figure 1) at each site between March and May of 2013. The team of individuals who assisted us in the field included undergraduate students at the University of New Hampshire, staff from the UNH Jackson Estuarine Laboratory, and volunteers from the community. We oriented the sampling transects perpendicular to the shoreline. Based on the photographs provided in Nettleton's 2011 report, we situated the "center" transects at each site to mimic the sampling locations he used as closely as possible. Using distances selected from a table of random numbers between 5 and 50 meters, we established outer transects on either side of the center transects. We marked points at -0.8 meters, 0 meters, +0.5 meters, +1.0 meters, and +1.5 meters relative to mean low low water (MLLW) on each transect.

To determine the elevation of MLLW at each site, we estimated the time at which low tide was expected to occur (NOAA Tide Tables). We also adjusted the heights of the low tides using the following tidal correction factors: 0.7% for Cedar Point, Wagon Hill Farm, and Lubblerland Creek and 0.75% for Depot Road. We confirmed the time of low tide with field observations. The research team then measured the elevation of the land at the edge of the current water level relative to a laser level positioned on shore using a laser receiver. Because the receiver was too distant to detect the laser beam emitted from the tripod at MLLW at the Lubberland Creek site, we used a top-down approach to estimate elevation instead. We used the water elevation at mean high water (MHW) for the month of May, 2013 as the mean high water mark with a water elevation of +9.6'. After establishing the MLLW or MHW baseline, the research team then marked sampling locations relative to the baseline using the laser level and receiver to find the chosen elevations. The extensive mudflats at the Lubberland Creek and Depot Road sites prevented me from establishing sampling points at MLLW at these sites. In the fall of 2013, we generated the latitude and longitude positions of each of the sampling points shown in Figure 1 using a Garmin etrex 20 GPS unit (APPENDIX A).

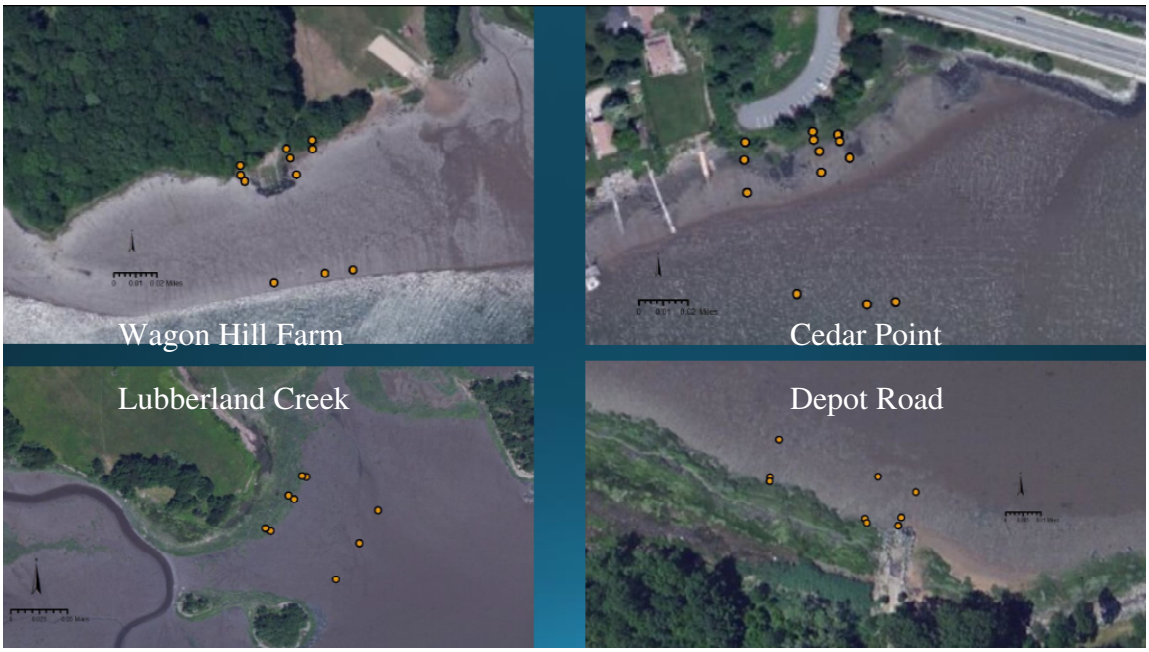


Figure 1. Dots depict points on 2013 macroalgal sampling transects established with GPS units. Sites shown are, from upper left to bottom right: Wagon Hill Farm, Cedar Point, Lubberland Creek, and Depot Road. Background imagery layer available in ESRI ArcGIS 10.1.

Data Collection

For each sampling event, we estimated the percentage of ground covered by each species/genus in a 0.25 m^2 quadrat and photographed the quadrat. We recorded the growth form (tubular or foliose) of *Ulva* specimens and attempted to separate *Gracilaria* specimens into species based on morphological features (Thomsen et al., 2006), but the data and results we present in this report assess *Ulva* and *Gracilaria* at the genus level. We then estimated percent cover and collected biomass from a smaller quadrat outside the original 0.25 m^2 quadrat, but at the same elevation. To begin sampling, we centered a 0.5 meter x 0.5 meter square of polyvinyl chloride (PVC) pipe above the sampling point, as marked with PVC pipe during site establishment. We excluded vegetation attached to substrata outside of the PVC quadrat from percent cover and biomass data collection. Once the quadrat was in place, we took a digital photograph of each sampling point. We recorded the species/genera present in each quadrat and categorized the percent of the quadrat area covered by each species as "<10%, "10%," or any multiple of 10% up to 100%.

On dates when we also collected biomass data, we laid out a 0.25 meter x 0.25 meter PVC square 2 meters east of each percent cover plot. We recorded percent cover for each species using the cover classes described above before collecting material from within the quadrat. To avoid resampling the same area, we collected the second biomass sample immediately north of the first, the third sample immediately east of the first, and the fourth sample immediately north of the third. So as to only measure live material, we discarded material that had lost its pigmentation.

While separating the species/genera in each sample in a laboratory, we removed dirt and other contaminants, such as snail eggs. We added any species/genera found during the cleaning

process that had not been noted in the field to the "1%" cover class for that sample point. I dried the sorted, clean material in an oven for 5 days at 60° C on foil sheets labeled with sample information before we measured its mass to the nearest hundredth of a gram. We then used the percent cover and corresponding biomass data to generate a linear regression expressing the relationship between cover and biomass for each of the most common species (those that occurred in four or more biomass samples).

Because we modified the methods used by Nettleton et al. (2011), it is important to note the key differences between our methods when comparing our datasets (Table 1). The two field sampling designs differ in that Nettleton et al. (2011) sampled 10 quadrats on one 10-meter transect at each elevation at each site, whereas we sampled one quadrat on each of three transects at each elevation at each site. We chose to establish more transects with fewer points on each transect so that we would have more independent replicates of measurements at each site and elevation in a simpler sampling design that would be easier to repeat over the long-term. We also opted to estimate percent cover in the field and report percent cover as a percentage of quadrat area rather than estimate percent cover using presence/absence data from a point intercept approach based on 25 points on a photograph as Nettleton et al. (2011) and Hardwick-Witman and Mathieson (1983) did.

Table 1. Comparison between methods used to collect macroalgal data in 2013 and methods used by Nettleton et al. (2011).

	Nettleton et al. 2011	**Cianciola et al. 2013**
Sites sampled	5	4 (excluded Sunset Farm)
Number and size of quadrats at each elevation at each site	10 / 0.25 m^2	3 / 0.25 m^2
Data collection at MLLW	All sites	Omitted for Southern Great Bay sites
Estimation of percent cover	Performed in laboratory using point-intercept method for 25 points on photographs of quadrats. Reported as percentage of points where taxon was found.	Performed in field for quadrats. Reported as percentage of quadrat area covered.
Estimation of biomass	Ten random 0.01 m^2 squares were sampled in each percent cover quadrat	A 0.0625 m^2 square was sampled outside of each percent cover quadrat

Statistical Analyses

A least-squares linear model was used to examine the main effects and interactions among the three factors describing total macroalgal cover in our sampling design: site, elevation, and month. Because we only collected data from MLLW at two sites, data from this elevation were excluded for model development. Although we collected percent cover data for saltmarsh vegetation, they were excluded from the model input so that we could use the model to isolate

patterns in macroalgal cover data. Following Nettleton et al. (2011), we first used an arcsine transformation to adjust the total percent of ground covered by macroalgae at each sampling point between 0.5 m above MLLW and 1.5 m above MLLW to better suit a linear model that describes normally-distributed data. The statistical software program JMP Pro 11.0 (SAS Institute, Inc., 2013) was used to construct a standard least squares model. After running the model with multiple data transformations, we concluded that the significance of the effects and interactions of site, elevation, and sampling month was fairly consistent across the transformations. However, the square root transformation had the most consistent performance across the range of the dataset, as demonstrated in residual plots for the modeled data points. For this reason, square root rather than arcsine transformed data were used in our analysis. We conducted Tukey's HSD tests at an alpha level of 0.05 to confirm the significance of and help interpret the effects in the least-squares linear model.

To test our ability to predict the mass of macroalgae present from percent cover data, we developed linear regressions by species/genus between these two variables. Logarithmic, reciprocal, and square root transformations were performed on the biomass data collected and percent cover data were regressed against each of the transformed biomass datasets for *Ulva*. Because residual plots showed that none of the transformations significantly improved model predictions, we generated a linear model for each taxon for which we had more than four data points using untransformed biomass data.

2. Random Sampling

The 2013 macroalgal research team followed the same protocol that was used to assess percent cover at the established long-term sampling sites to assess percent cover by species/genus at 155 random points across the Great Bay Estuary. U.S. EPA's Western Ecology Division (Corvallis, OR) generated a list of 200 random points using a map of the Estuary produced by the New Hampshire Department of Environmental Services. The map defined our sample frame to include the tributaries of the estuary within New Hampshire up to the head of tide and all portions of the estuary that are intertidal and subtidal, but less than 2 meters deep at MLLW. In this sample frame, we intended to capture all parts of the Estuary that receive enough light to support rooted or benthic photosynthetic organisms. We transferred the coordinates of the random points into two GPS units for use in the field.

In the six weeks following the spring tide on August 24, 2013, teams of professional scientists and volunteers visited each random point once. We accessed the sampling points by walking from shore or kayaking when possible. Most points, however, were accessed via motorboat, which required that samplers enter the water. Navigating to the exact locations of the sampling points presented a challenge, especially when traveling by motorboat. When the GPS unit in use indicated that the research team was within 20 feet of the targeted random point, the team dropped a PVC quadrat directly below the GPS unit or over the side of the boat. Quadrats used on motorboats had weights and floats attached to prevent them from moving once they were set in place.

Teams used standardized field sheets (APPENDIX E) to collect data according to the percent cover estimate protocol developed for our transect sampling approach. One team member centered a 0.5 meter x 0.5 meter square of polyvinyl chloride (PVC) pipe above the sampling

point and excluded vegetation attached to substrata outside of the PVC quadrat. A second team member then categorized the percent of the quadrat area covered by each species as "< 10%" or any multiple of 10% up to 100%. The data sheets also allowed researchers to note observations of the weather, water depth, and vegetation surrounding the quadrat.

At the outset, the goal of the random sampling study was to collect data from 50 points of each of four cover types: macroalgae, eelgrass, mudflat, and salt marsh. Achieving this goal would provide enough data to reasonably differentiate the aforementioned cover types in remote sensing analyses. The research teams visited 175 of the 200 proposed random sampling points, but were unable to collect data at 19 of them. It was originally intended that researchers would visit additional randomly selected points beyond the first 200 points on the randomly-generated list to replace points where we did not collect data due to constraints such as unsafe water depths or dangerous water currents (near the Piscataqua River Bridge) for snorkelers. Researchers were not equipped to scuba dive for this study.

Three factors lead me to conclude the random sampling study, short of the original goal, on September 13, 2013: 1) the data collected were meant to be representative of conditions on the ground when Kappa Mapping, Inc. of Bangor, ME took aerial photographs of the Bay for the Piscataqua Region Estuaries Partnership on August 24, 2013; 2) the limited availability of researchers and boats during low tide constrained field sampling; and 3) the third round of transect sampling was scheduled to begin the following week, as described in the methods for the transect sampling approach. Furthermore, reviewing the incoming random sampling data, it became clear that continuing to work through the list of randomly selected points would use a lot of time and resources to locate more points in the Estuary's mudflats, which we had already more than sufficiently covered. With the resources available for this project, it was impracticable to acquire 50 data points for each of the four cover types. For example, saltmarsh habitat occurred uncommonly in the set of randomly generated points (12 of the 155 points where data were collected).

3. Isotope Ratio Analysis

To perform isotope ratio analyses, we collected tissue from *Ulva* specimens (foliose form) from 20 sites located in Great Bay Estuary, tidal rivers that feed into the Bay, and the Gulf of Maine at the mouth of the Estuary (Figure 2) between September and October of 2013. In rocky intertidal habitats, we collected attached material, but most of the samples from mudflats or subtidal areas were adrift. We attempted to collect samples from locations that were well-distributed around Great Bay Estuary, but could not find *Ulva* in several tidal rivers or at convenient access points on the mainstem of the Piscataqua River. In the laboratory, dirt and debris were cleaned from the samples, and the samples dried in an oven for 3 days at 60° C. The dried samples were crushed using a sterilized mortar and pestle and 2 milligrams of each sample were measured into small glass vessels for shipment to the Boston University (BU) Stable Isotope Laboratory.

The BU Laboratory used continuous flow isotope ratio mass spectrometry to determine the $^{15}N : ^{14}N$ and $^{13}C : ^{12}C$ ratios of the tissue samples. To provide quality assurance and quality control, the BU Laboratory analyzed peptone and glycine as standards and ran the analysis twice for every third sample (APPENDIX F). The mean ^{15}N value was calculated for samples that were analyzed twice. Linear regressions were run in JMP Pro 11.0 (SAS Institute, Inc., 2013) to test

the significance of the relationship between the ^{15}N and ^{13}C signatures of a sample and between the ^{15}N signature of a sample and the distance between the sample site and the mouth of Portsmouth Harbor.

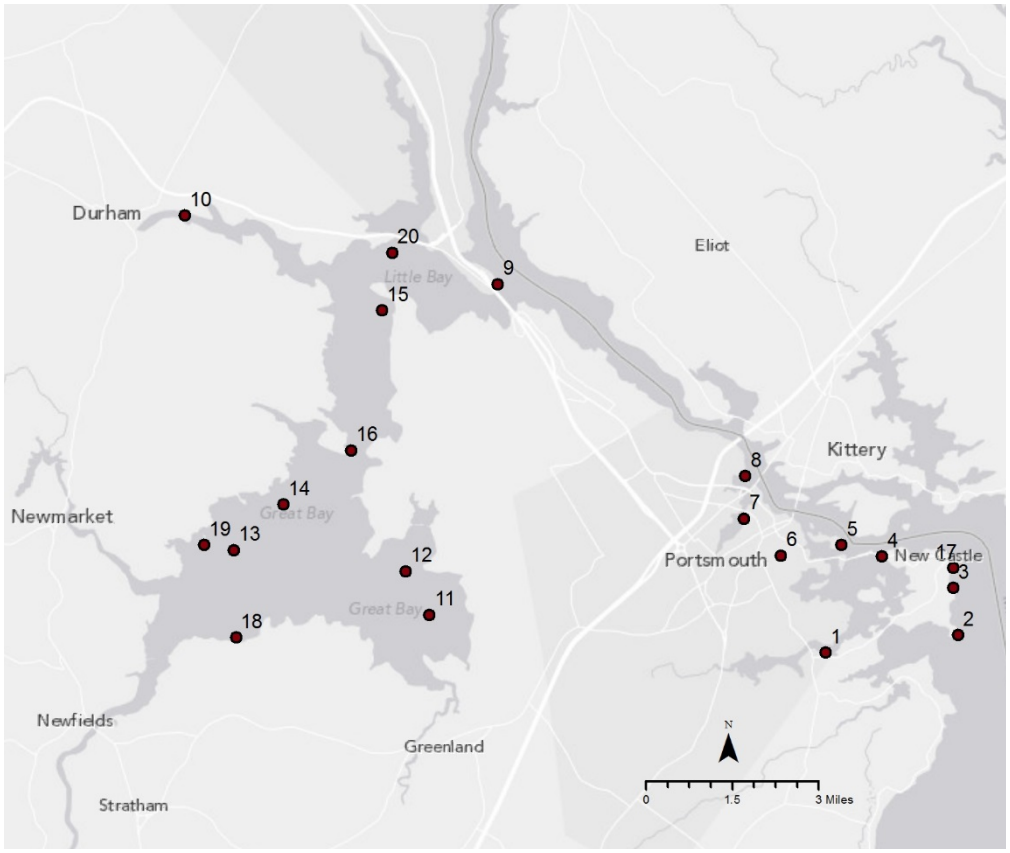


Figure 2. Map of collection sites for *Ulva* samples analyzed for nitrogen isotope ratios in fall of 2013. Background imagery is ESRI basemap from ArcMap 10.1.

Results

1. Transect Sampling

Interactions between Variables Determining Total Macroalgal Cover

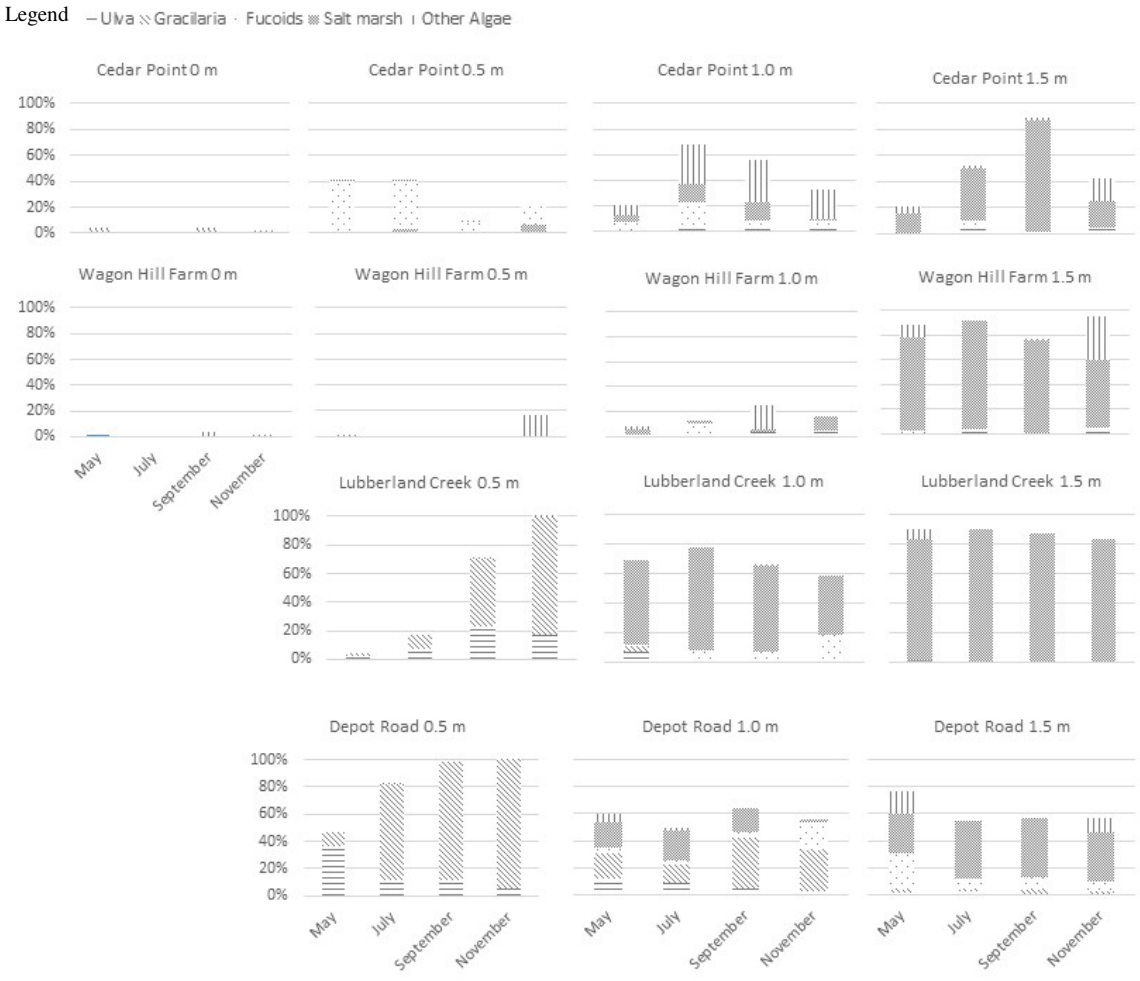
The percentage of ground covered by five major groups of intertidal vegetation over time at each of the elevations sampled at each site is summarized in Figure 3. We included saltmarsh grasses as a category on the graphs to illustrate the fact that the lower levels of macroalgae at 1.0-1.5 m above MLLW should not be interpreted as an indication of lower primary productivity at these elevations. The sites ranked from highest to lowest overall vegetated cover are as follows: Lubberland Creek, Depot Road, Cedar Point, and Wagon Hill Farm. Ranking the sites from highest to lowest macroalgal cover reorders them as follows: Depot Road, Cedar Point, Lubberland Creek, and Wagon Hill Farm. In general, macroalgal cover peaked between 0.5 m and 1 m above MLLW, with bare ground being predominant at 0 m above MLLW and saltmarsh vegetation dominating at 1.5 m above MLLW. The effect of sampling month on macroalgal cover did not follow a consistent trend across any one site or elevation.

Examining the main effects and interactions of the three factors describing total macroalgal cover in our sampling design by means of a least-squares linear model shows which of them are most important in describing patterns in macroalgal abundance and distribution in Great Bay Estuary. The model for total macroalgal cover is significant at the $p < 0.01$ level, has an R^2 value of 0.72, and has a root mean square error (RMSE) of 24%. Effects due to site, elevation, and month are significant at the $p < 0.01$ level, as are the interaction between site and elevation and the interaction between all three factors (Table 2).

Table 2. Significance of effects due to site, elevation, and sampling month in least-squares linear model of macroalgal cover in Great Bay Estuary.

Variable	Sum of squares	F ratio	ANOVA Prob > F
Site	4.02	23.39	< 0.0001
Elevation	1.07	9.32	0.0002
Month	0.73	4.23	0.0074
Site x elevation	3.76	10.93	< 0.0001
Site x month	0.68	1.32	0.24
Elevation x month	0.63	1.83	0.10
Site x elevation x month	2.95	2.86	0.0005

Figure 3. Percent of sample area covered by *Ulva* spp., *Gracilaria* spp., fucoids (*Ascophyllum nodosum* and *Fucus vesiculosus*), salt marsh (*Spartina alterniflora*, *Spartina patens*, *Distichlis spicata*), and arranged by sampling elevation and month at each site. Less common macroalgae are combined into a single category that includes *Ceramium virgatum* Roth, *Cladophora sericea* (Hudson) Kützing, *Chondrus crispus* Stackhouse *Heterosiphonia japonica* Yendo, *Polysiphonia stricta* (Dillwyn) Greville, and *Pylaiella littoralis* (L.) Kjellman. Error bars not shown in order to maintain readability.



TheTukey's HSD results confirmed the apparent main effects due to site and elevation in (Figure 3). The Depot Road, Cedar Point, and Wagon Hill Farm sites had significantly different total macroalgal cover, but total macroalgal cover at the Lubberland Creek site did not differ significantly from that at the Cedar Point or Wagon Hill Farm sites. At + 0.5 m, mean total macroalgal cover was significantly higher than at + 1.0 or + 1.5 m, but mean total macroalgal cover did not differ significantly between the two higher elevations. The effect of month alone showed that mean total macroalgal cover was highest in November and lowest in May. Although mean total macroalgal cover values in May and July were significantly lower than in November, mean total macroalgal cover in September was not significantly different from mean total macroalgal cover in any other month.

Blooms of drift macroalgae drove the significant interaction between site and elevation. At + 0.5 m, the Lubberland Creek and Depot Road sites had significantly greater cover of macroalgae, which was dominated by *Gracilaria* and *Ulva* (Figure 3). The Cedar Point and Depot Road sites had moderate macroalgal cover at + 1.0 m, but attached macroalgae such as *Cladophora* Kützing spp. dominated macroalgal cover at the Cedar Point site, whereas *Gracilaria* dominated macroalgal cover at the Depot Road site (Figure 3). Macroalgal cover at the Lubberland Creek and Wagon Hill Farm sites was low at + 1.0 m;

Examining the main effects and interactions of the three factors describing total macroalgal cover in our sampling design by means of a least-squares linear model shows which of them are most important in describing patterns in macroalgal abundance and distribution in Great Bay Estuary. The model for total macroalgal cover is significant at the $p < 0.01$ level, has an R^2 value of 0.72, and has a root mean square error (RMSE) of 24%. Effects due to site, elevation, and month are significant at the $p < 0.01$ level, as are the interaction between site and elevation and the interaction between all three factors (Table 2).

Table 2. Significance of effects due to site, elevation, and sampling month in least-squares linear model of macroalgal cover in Great Bay Estuary.

Variable	**Sum of squares**	**F ratio**	**ANOVA Prob > F**
Site	4.02	23.39	< 0.0001
Elevation	1.07	9.32	0.0002
Month	0.73	4.23	0.0074
Site x elevation	3.76	10.93	< 0.0001
Site x month	0.68	1.32	0.24
Elevation x month	0.63	1.83	0.10
Site x elevation x month	2.95	2.86	0.0005

Figure 3 shows that saltmarsh dominated this elevation at the Lubberland Creek site. Data collected from + 1.5 m offer the least explanatory power in the site by elevation interaction, likely due to grass cover in the salt marshes. At + 1.5 m, macroalgal cover was low at all of the sites, with moderate fucoid cover on one of the transects at the Depot Road site (Figure 3).

The significance of the three-way interaction in the model shows that the relationship between site and elevation changed significantly over sampling months. Including sampling month as an independent variable in the model does help explain differences between estimates of mean total macroalgal cover for some subsets of sample points. For example, at + 0.5 m, total macroalgal cover increased dramatically at the two southern sites over the growing season, but not at the two northern sites (Figure 3). Although sampling month seems to provide some explanatory power for differences between mean total macroalgal cover estimates in the entire dataset, the weakness of its effect suggests that sampling month only explains the differences in mean total macroalgal cover between specific combinations of elevations and sites.

Neither the interaction between sampling month and elevation nor the interaction between sampling month and site are significant effects in the model ($p > 0.1$). The interaction between sampling month and elevation is not significant in separate standard least squares models of total macroalgal cover at each site, either ($p > 0.1$). The lack of significance of the interactions between sampling month and site or elevation suggests that sampling at different times of the year would not be necessary if one wanted to characterize total macroalgal cover at different sites at one elevation or at different elevations at one site in Great Bay Estuary. Sampling at different times of the year only becomes critical when one wants to see the dynamics of total macroalgal cover through the full spatial extent (various elevations across sites) of the Great Bay estuarine system. As mentioned in the discussion of algal blooms, the contributions of various taxa to the total macroalgal cover at a sampling point does change over the course of the year, with *Ulva* predominating in spring months and *Gracilaria* predominating from late summer into early fall. Thus it is possible to devise a sampling design that captures peak cover throughout the spatial extent of Great Bay Estuary for a taxon of interest without sampling at different times of the year.

To facilitate comparisons between the 2008-2010 and 2013 datasets, we present the mean percent cover values for the species or morphological “forms” of macroalgae or saltmarsh grasses observed in each sampling month at each of the four transect sites in APPENDIX B.

Biomass Sampling

Although we designed the biomass sampling protocol to support the percent cover transect sampling, the biomass data collected in and of themselves provide a basis for characterizing the vegetation of the sites studied. We summarized the biomass data as mean monthly cover values for each site (

Table 3). In general, high levels of macroalgal biomass occurred where and when the biomass of saltmarsh vegetation was low, as is also evident in the percent cover data. The Depot Road site is an exception in that both its macroalgal and saltmarsh biomass were high in July, and the site supported high masses of both fucoids and other macroalgae in September and November.

Because it is of interest to quantify the spread of the non-native invasive *Gracilaria vermiculophylla* (Ohmi) Papenfuss in Great Bay Estuary, we tabulated the *Gracilaria* biomass data collected by species as distinguished based on morphological features (Thomsen et al., 2006). The data collected show that the biomass of *Gracilaria vermiculophylla* consistently exceeded the biomass of *Gracilaria tikvahiae* McLachlan at the Depot Road and Lubberland Creek sites throughout the sampling period (Table 4). Although the percent cover data show that drift macroalgae were visually predominant in Great Bay Estuary in 2013, the denser, long-lived fucoids still dominate the macroalgal biomass of sites that have rocky intertidal habitats (Table 3).

Table 3. Biomass of macroalgae and saltmarsh vegetation averaged across elevations at each site in each sample month.

Site	Month	Macroalgae (g/m^2)	Fucoids (g/m^2)	Other Macroalgae (g/m^2)	Saltmarsh (g/m^2)
Cedar Point	May	471.1	448.4	9.6	1.9
	July	94.4	86.9	15.7	32.8
	September	14.0	2.1	25.3	47.2
	November	124.4	92.1	19.3	34.9
Depot Road	May	54.9	1.4	53.4	44.6
	July	504.8	455.1	49.7	103.1
	September	447.3	291.2	156.1	69.9
	November	195.0	88.3	106.6	25.3
Lubberland Creek	May	2.0	0.0	2.0	92.5
	July	27.1	24.7	2.5	208.9
	September	29.9	3.2	26.7	180.4
	November	116.9	25.4	91.5	136.0
Wagon Hill Farm	May	47.1	14.5	32.6	8.3
	July	18.5	18.5	0.0	79.8
	September	966.8	965.9	0.9	0.0*
	November	90.9	83.1	7.8	19.1

*Saltmarsh vegetation was present at Wagon Hill Farm in September, but did not fall in the biomass sampling quadrats that month due to the patchiness of the salt marsh at this site.

Table 4. *Gracilaria* biomass at Depot Road and Lubberland Creek separated by species based on morphological features described by Thomsen et al. (2006).

	Depot Road		Lubberland Creek	
	G. tikvahiae (g/m^2)	G. vermiculophylla (g/m^2)	G. tikvahiae (g/m^2)	G. vermiculophylla (g/m^2)
May	2.0	19.6	0.0	0.6
July	12.6	20.9	0.1	1.7
September	24.5	96.2	1.1	20.3
November	2.8	92.4	15.8	31.7

Linear Regressions to Predict Biomass from Percent Cover

Thirteen species of macroalgae, three additional morphological "forms" of macroalgae identified at the genus level, and three species of saltmarsh grasses were present in the biomass samples. As mentioned previously, percent cover for all *Gracilaria* species are reported as one value, and percent cover for *Ulva* species are reported as either "tubular" or "foliose" form rather than by

species. The presence of epiphytes in the biomass samples was noted when they were encountered, but they are excluded from the results presented in this report. The objective of this study was to monitor macroalgae, not microalgae, and the visible microalgae growing on substrata or other plants never reached 1% coverage of the area in the 0.0625 m^2 quadrat when percent cover estimates were made in the field.

Linear regressions (Table 5) were used to predict biomass in grams as a function of percent cover for the eight species or "forms" collected more than four times in 2013 (APPENDIX D). All of the models are significant at the $p < 0.01$ level, but the error associated with the biomass predictions ranges from 0.4 g for *Pyliella littoralis* to 9.5 g for *Fucus vesiculosus*. The linear regressions were used to estimate how much biomass was present in the 0.25 m^2 quadrats so that we could still have biomass data for the 0.25 m^2 percent cover quadrats without impacting the cover and biomass that would be present when we returned for the next sampling event. In this way, the same locations can be used to repeatedly assess changes in macroalgal abundance.

Table 5. Regression models for common macroalgal species based on data collection in Great Bay Estuary, NH in 2013.

Genus/Species	Model	N	r^2	RMSE	ANOVA Prob > F
Gracilaria spp.	Mass = -0.019 + 9.34 (cover)	28	0.82	1.6 g	<0.0001
Ulva (foliose)	Mass = -0.20 + 6.96 (cover)	23	0.69	1.2 g	<0.0001
Fucus vesiculosus	Mass = 0.96 + 38.94 (cover)	29	0.54	9.5 g	<0.0001
Pyliella littoralis	Mass = -0.37 + 8.69 (cover)	10	0.91	0.4 g	<0.0001
Cladophora sericea	Mass = -0.54 + 10.39 (cover)	11	0.70	1.8 g	0.0001
Ceramium virgatum	Mass = -0.067 + 13.29 (cover)	7	0.95	1.3 g	0.0002

The tables in APPENDIX C show the mean biomass values determined from the percent cover values estimated in the field for the 0.25 m^2 plots (APPENDIX B) using the linear regressions described above. Graphs of the linear regression models are included in APPENDIX D.

2. Random Sampling

Each of the 155 points sampled in 2013 were classified as "macroalgae," "eelgrass," "saltmarsh," or "bare" according to the cover type that dominated the quadrat area at the sampling point. If no vegetation type occupied at least 10% of the quadrat area, the point was classified as "bare." The one "freshwater marsh" point on the Salmon Falls River was below the dam, which we treated as the head of tide, but it was evident that the species composition at this point was not impacted by the presence of saltwater. Any future environmental monitoring efforts for Great Bay Estuary that intend to assess vegetation that is adapted to saltwater influence should avoid this section of the Salmon Falls River. We summarized the prevalence of each cover type and the land area represented by each cover type in our dataset in Table 6. We were unable to collect data at 38 sites that could have been sampled; these are classified as "Not Sampled." We removed the 7 sites we located, but found to be below the photic zone from the calculations, leaving estimates based on 193 samples. A fifth of the sample area had >10% macroalgal cover and < 10% eelgrass or saltmarsh cover.

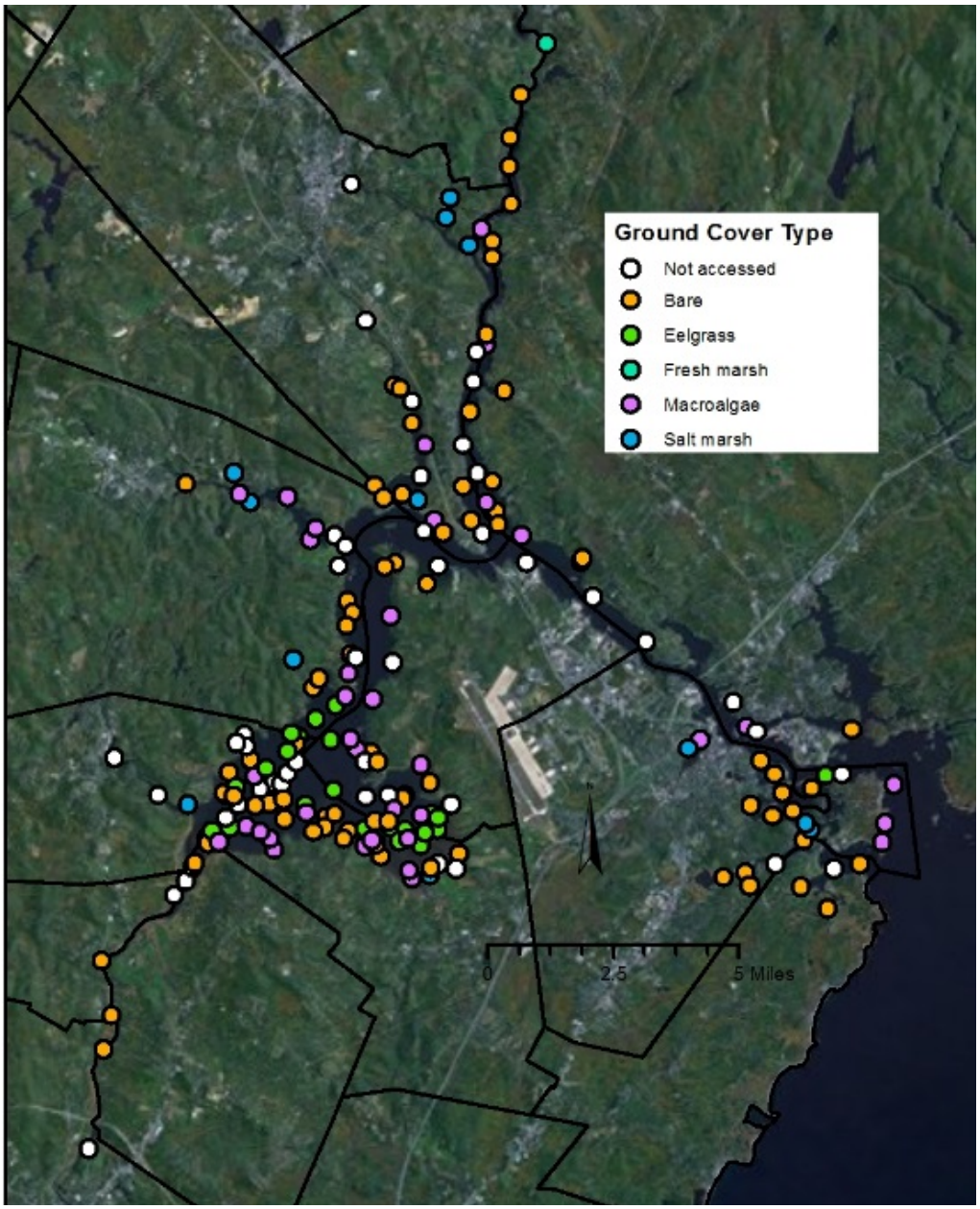


Figure 4. Map of 200 random sampling points with the 155 points accessed in August and September of 2013 classified by cover type.

Table 6. Frequency of the four cover types assessed within the 193 random sample points within the sample frame.

Cover Type	No. Sites	Percent of Sites	Area of Sample Frame in Estuary (Acres)
Eelgrass	24	13	1105
Macroalgae	38	19	1615
Saltmarsh	12	6	510
Bare	81	42	3570
Not sampled	38	20	1700

Each of the taxa that the 2013 macroalgal research team found at the random sampling points and the frequency at which we observed each taxon are listed in Table 7. Eelgrass was found most frequently, at 19% of the points. The five most common species/genera of macroalgae among the points we sampled in Great Bay Estuary were *Gracilaria* spp., *Ulva* spp., *Ascophyllum nodosum*, *Fucus vesiculosus*, and *Pyliella littoralis*. The mean level of macroalgal cover across all 155 data points was 9.2% ± 22.5 SD. When we excluded points where no macroalgae were found, the mean level of macroalgal cover increased to 11.7% ± 24.8 SD.

Table 7. Genera and species of plants and macroalgae found at random sampling sites in August and September of 2013.

Scientific Name	Common Name	Number of Sites Where Present	Percent of Sites Sampled
Zostera marina	Eelgrass	29	18.6%
Gracilaria spp.	(Red algae)	25	16.0%
Ulva (foliose)	Sea lettuce	17	10.9%
Spartina alterniflora	Smooth cordgrass	9	5.8%
Ascophyllum nodosum	Knotted wrack	4	2.6%
Vaucheria sp.	(Green algae)	3	1.9%
Chondrus crispus	Irish moss	2	1.3%
Fucus vesiculosus	Bladderwrack	2	1.3%
Distichlis spicata	Spike grass	2	1.3%
Ulva (tubular)	Gut weed	1	0.6%
Cladophora sericea	(Green filamentous algae)	1	0.6%
Heterosiphonia japonica	Siphoned Japan weed	1	0.6%
Spartina patens	Salt hay	1	0.6%
Schoenoplectus maritimus	Bulrush	1	0.6%
Pontedieria cordata	Pickerelweed	1	0.6%
Limonia carolinianum	Sea lavender	1	0.6%

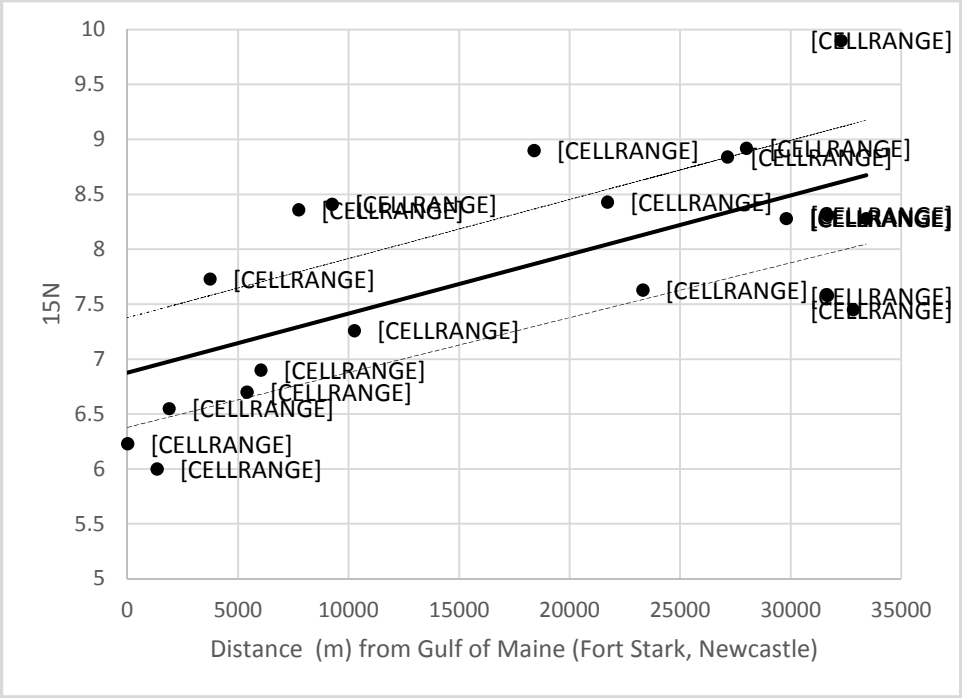
3. Isotope Ratio Analysis

The sites where *Ulva* tissue was collected is shown along with the isotope ratio mass spectrometry results (Table 8). A significant trend of increasing ^{15}N abundance with increasing distance from the Gulf of Maine is evident in Figure 5 and is confirmed by the linear regression analysis ($p < 0.01$). The cluster of samples in Figure 5 that have low ^{15}N relative to their distance from the ocean (around 3000 meters from Fort Stark) includes the four samples collected from eelgrass beds (11-14). Sites that show high ^{15}N relative to their distance from the ocean include sites 1, 6, 7, 9, and 19. Combined sewer overflows, highly developed watersheds, and effluent from wastewater treatment plants likely raise the availability of ^{15}N at sites 6, 7, and 9, respectively.

Table 8. Collection sites for *Ulva* samples analyzed for nitrogen and carbon isotope ratios. ^{15}N and ^{13}C values are reported relative to the natural occurrence of the isotopes.

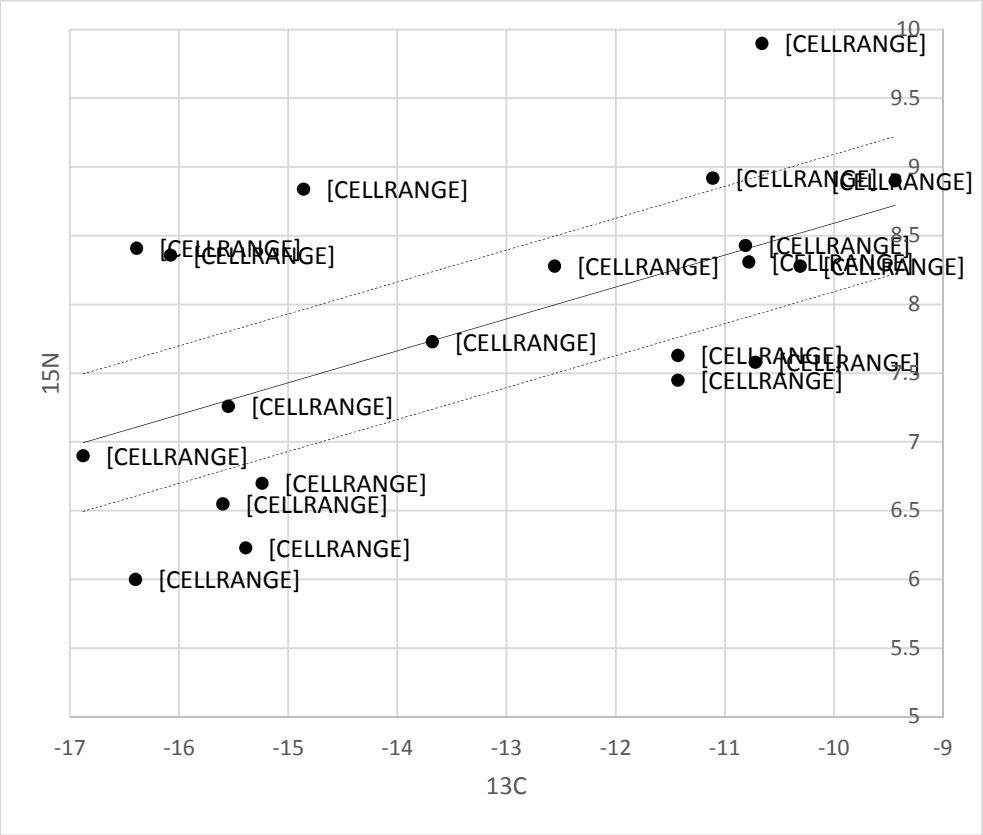
Site	Site Name	Town	Description	^{15}N	^{13}C
1	BG's Boathouse	Portsmouth	Intertidal	7.73	-13.68
2	Fort Stark	New Castle	Intertidal	6.23	-15.39
3	New Castle coast (1)	New Castle	Intertidal	6.00	-16.4
4	Downstream of WWTP	Portsmouth	Intertidal	6.70	-15.24
5	Peirce Island	Portsmouth	Subtidal	6.90	-16.88
6	South Mill Pond	Portsmouth	Subtidal	8.31	-16.08
7	North Mill Pond	Portsmouth	Intertidal	8.46	-16.39
8	Freemans Point	Portsmouth	Intertidal	7.73	-15.55
9	Dover Point	Dover	Intertidal	8.90	-9.44
10	WWTP on Oyster River	Durham	Intertidal	8.92	-11.11
11	Sunset Farm	Greenland	Eelgrass bed	7.45	-11.43
12	Herods Cove	Newington	Eelgrass bed	7.58	-10.72
13	North of Lubberland Creek	Newmarket	Eelgrass bed	8.31	-10.78
14	South of Jackson Estuarine	Durham	Eelgrass bed	8.28	-10.31
15	Cove south of Fox Point	Newington	Subtidal	7.63	-11.43
16	Cove at Jackson Estuarine Lab	Durham	Intertidal	8.84	-14.86
17	New Castle coast (2)	New Castle	Intertidal	6.55	-15.6
18	Great Bay Discovery Center	Greenland	Intertidal	8.28	-12.56
19	Lubberland Creek Nature Preserve	Newmarket	Intertidal	9.90	-10.66
20	Cedar Point	Durham	Subtidal	8.43	-10.81

Figure 5. ^{15}N values for *Ulva* samples collected around Great Bay Estuary in fall of 2013 as a function of distance from the Gulf of Maine. Label numbers correlate to sites listed in Table 8. The dashed lines encompass the region +/- 0.5 SD around the line of best fit.



Carbon isotope data are often used as a baseline or control for the interpretation of nitrogen isotope data. The abundances of ^{13}C in the *Ulva* tissue collected were used to generate Figure 6. As opposed to nitrogen isotope ratios, which are reported based on ^{15}N enrichment, carbon isotope ratios are reported based on depletion of ^{13}C relative to a standard ratio, and, as a result, are negative. The depletion of ^{13}C was generally greater in *Ulva* tissue that had lower ^{15}N enrichment and was collected closer to the Gulf of Maine.

Figure 6. ^{15}N values for *Ulva* samples collected around Great Bay Estuary in fall of 2013 as a function of ^{13}C values. Label numbers correlate to sites listed in Table 8. The dashed lines encompass the region +/- 0.5 SD around the line of best fit.



Discussion

1. Transect Sampling

The transect-based macroalgal sampling confirms the increased abundance of macroalgae in Great Bay Estuary reported by Nettleton et al. (2011) and suggests that the abundance of macroalgae in Great Bay Estuary is comparable to their abundance in other estuaries in the northeastern U.S. Most published assessments of macroalgal cover outside of Great Bay Estuary have used approaches that assign cover classes to large sample units (e.g. Lyons et al., 2009 and Raposa et al., 2011) as opposed to conducting transect-based sampling. Our observations of average macroalgal cover in Great Bay Estuary in 2013 correspond to cover classes 1-2 on a typical 5-point scale designed to score sites encompassing an area on the order of 1 km^2. Previous studies have observed similar levels of macroalgal cover in other estuaries in the northeastern U.S. For example, Lyons et al. (2009) reported a mean cover class greater than 2 for 5 of the 40 sites they studied on Cape Cod National Seashore, MA. Similarly, 90% of the scores Raposa et al. (2011) assigned to sites on Prudence Island, RI were between 0 and 2, but 4% of the scores were 4 or 5.

Bloom Macroalgae

Due to their interest in algal blooms, Nettleton et al. (2011) isolated their *Ulva* and *Gracilaria* data for further analysis. Comparisons with 2013 data show similar patterns between the sites in northern and southern parts of Great Bay Estuary. However, 2013 data also suggest that point-intercept sampling of photographs of quadrats may provide a different assessment of percent cover than visually estimating the percentage of the entire quadrat area that is covered while sampling in the field.

In a site-by-site comparison of percent cover, mean *Ulva* cover (including both foliose and tubular forms) was lower at all sites in 2013 than in 2008-2010 (Figure 7). The tubular form dominated *Ulva* cover at the northern sites (Wagon Hill Farm and Cedar Point) and in the saltmarsh samples at all sites (all samples at + 1.5 m and some of the samples at + 1.0 m), whereas the foliose form dominated the samples collected at + 0.5 m at the southern sites (Lubberland Creek and Depot Road). Nettleton et al. (2011) did not observe the tubular form at the southern sites in 2008-2010, and it never exceeded 10% cover at these sites in 2013.

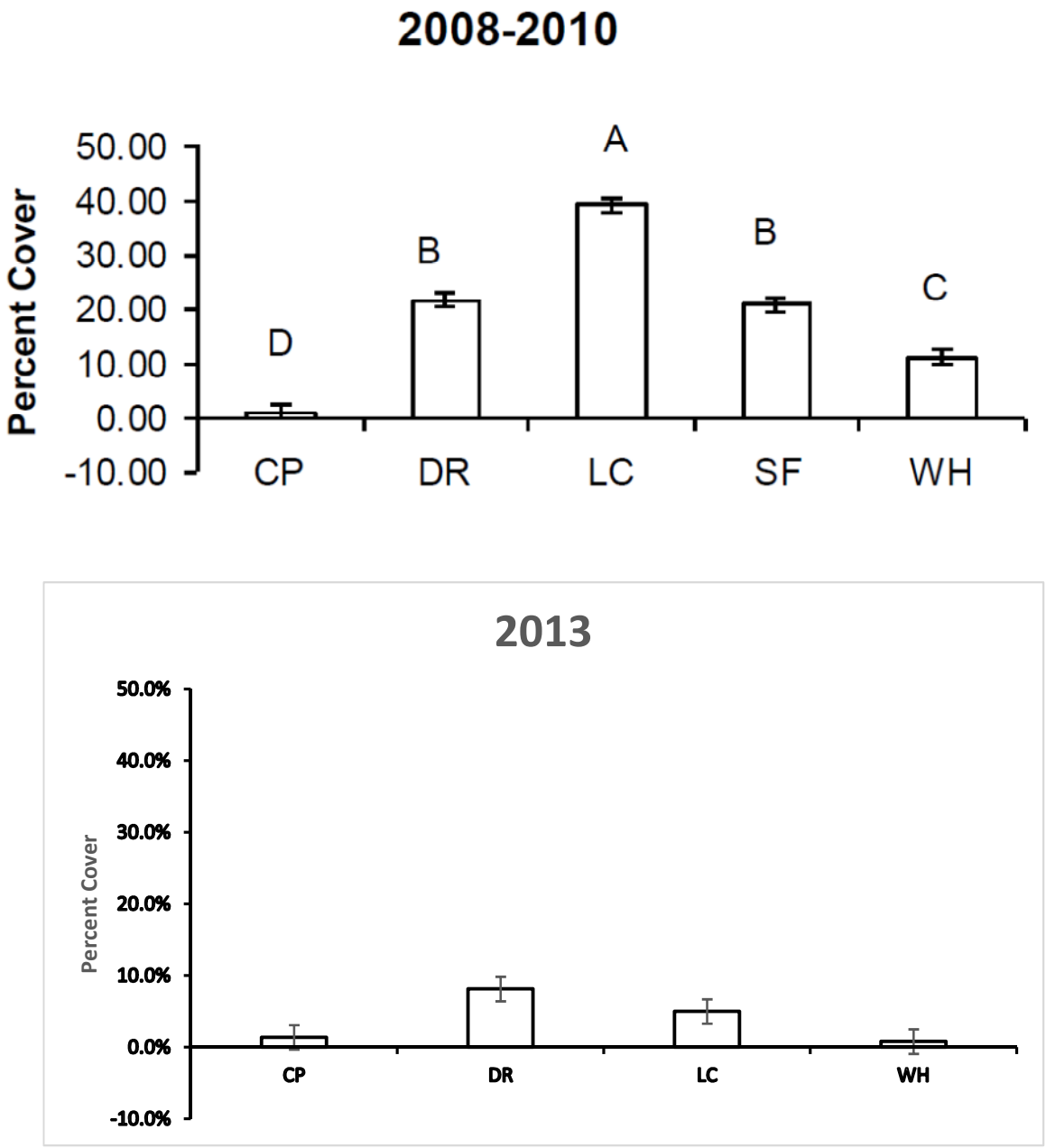


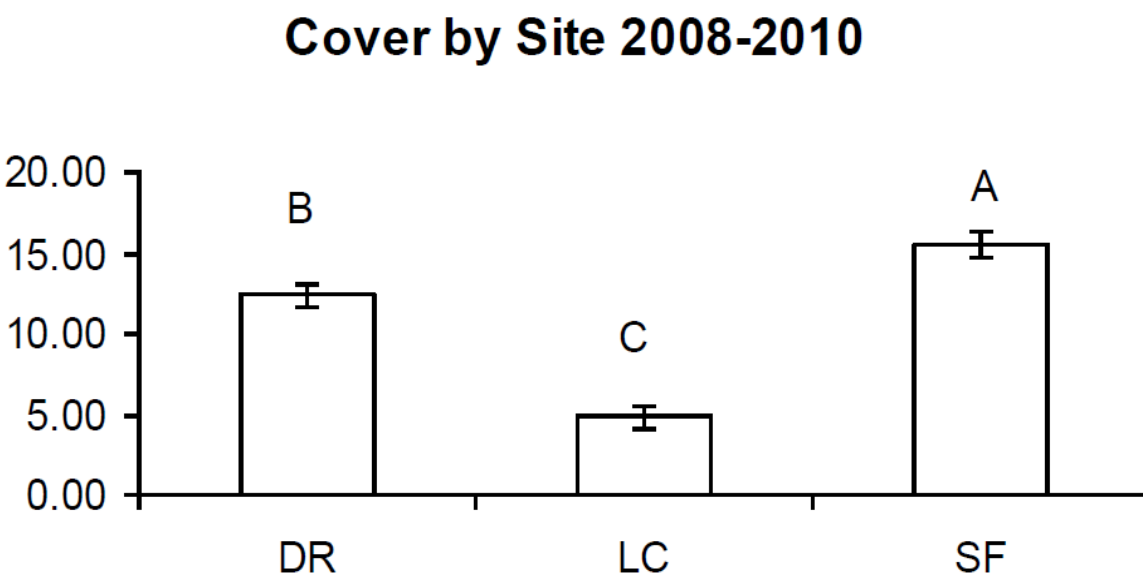
Figure 7. Mean percent cover of *Ulva* in 0.25 m^2 quadrats at all sites included in the 2008-2010 and 2013 macroalgae transect sampling. The 2008-2010 figure was first published by Nettleton et al. (2011).

Both the 2008-2010 datasets and the 2013 datasets show higher *Ulva* cover at the sites in southern Great Bay (Depot Road and Lubberland Creek) than in northern Great Bay. The mean *Ulva* cover Nettleton et al. (2011) observed at Lubberland Creek, 39%, is significantly higher than the mean *Ulva* cover at any other site in his study or in 2013. A paired t-test comparing the monthly mean *Ulva* cover values at Lubberland Creek in 2009 and 2013 shows that the annual mean *Ulva* cover values are significantly different ($p < 0.05$). In 2013, the mean *Ulva* cover of 8% observed at Depot Road was similar to the mean *Ulva* cover of 5% observed at Lubberland Creek.

The highest mean cover value for *Ulva* at one site during one sampling period was 90% at Lubberland Creek in November of 2008 (Nettleton et al., 2011). It is important to recognize, though, that the percent cover values Nettleton et al. (2011) report are based on presence/absence observations for each taxon for 25 points in each quadrat. To achieve such a high mean cover value using the 2013 data collection method would have necessitated that no more than five samples had less than 90% *Ulva* cover, and only if every other sample was completely covered by *Ulva* could one sample have less than 10% cover. Because the *Ulva* cover at Lubberland Creek in November of 2013 varied between 0 and 10% in the saltmarsh and was about 20% in the mudflats, the mean *Ulva* cover was only 6%.

Nettleton et al. (2013) report that the Asiatic red alga, *Gracilaria vermiculophylla*, was first collected in Great Bay Estuary at Dover Point in 2003. Although *Gracilaria vermiculophylla* has been moving up the Atlantic coast from Virginia since the 1990s, it has not yet been documented as far north as Maine (Nettleton et al., 2013). The biomass data collected from the Lubberland Creek and Depot Road sites show significant blooms of *Gracilaria vermiculophylla* (Table 4). However, because of the limited reliability of using morphological features to distinguish this non-native species from the native *Gracilaria tikvahiae* and the financial burden of sequencing DNA from all samples from all sites, we followed the precedent set by Nettleton et al. (2011) and assessed the cover of *Gracilaria* at the genus level.

In 2013, the *Gracilaria* bloom was more severe than it was in 2008-2010 (Figure 8), likely due to expansion of the *Gracilaria vermiculophylla* population. The highest mean *Gracilaria* cover in Nettleton's 2008-2010 study was observed at Sunset Farm. We did not collect samples at Sunset Farm in 2013, but the *Gracilaria* cover at Depot Road exceeded the *Gracilaria* cover at Lubberland Creek again in 2013, as it did in 2008-2010. However, the mean *Gracilaria* cover was higher at both sites in 2013 than in 2008-2010. Mean *Gracilaria* cover at Depot Road was 12% in 2008-2010 and 31% in 2013. At Lubberland Creek, *Gracilaria* cover was 5% in 2008-2010 and 12% in 2013. However, paired t-tests comparing the mean monthly *Gracilaria* cover values at Lubberland Creek and Depot Road from 2009 and 2013 only show significantly higher *Gracilaria* cover at Depot Road ($p < 0.05$). In both 2008-2010 and in 2013, *Gracilaria* cover was negligible at Wagon Hill Farm and Cedar Point. There were no observations of *Gracilaria* in 2008-2010 at either site, none at Wagon Hill Farm in 2013, and a mean value of 0.9% *Gracilaria* cover at Cedar Point in 2013.



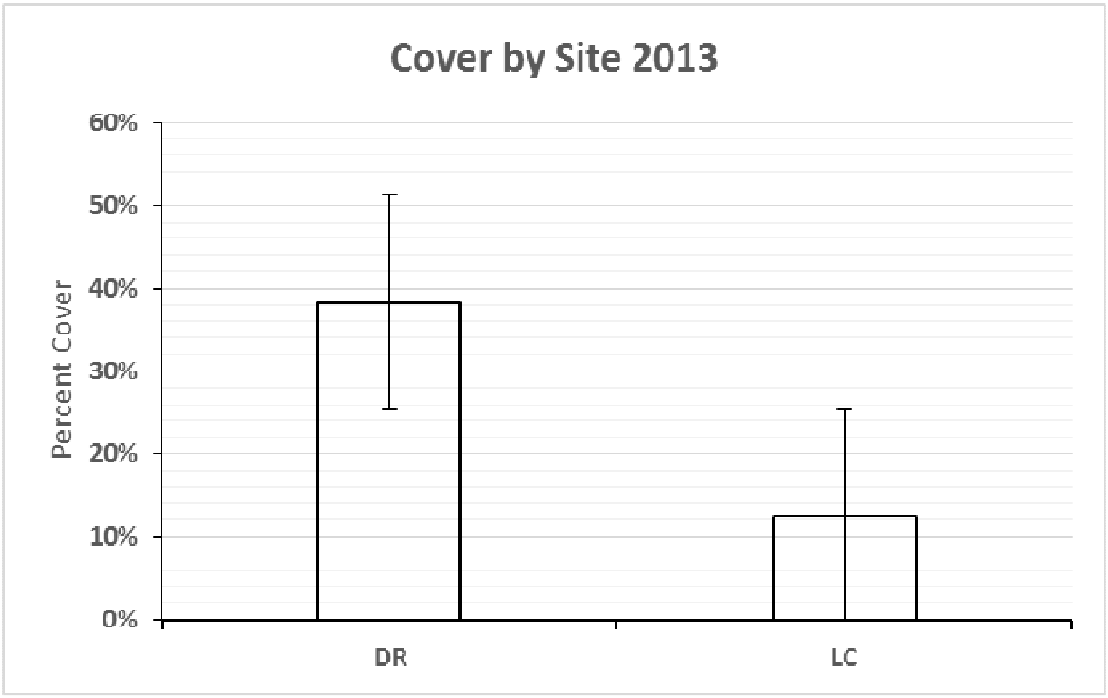


Figure 8. Mean percent cover of *Gracilaria* in 0.25 m^2 quadrats at Depot Road, Lubberland Creek, and Sunset Farm (Southern Great Bay) in 2008-2010 and 2013. Sunset Farm was not sampled in 2013. The 2008-2010 figure was first published by Nettleton et al. (2011).

As shown by the significant three-way interaction in the linear model of total macroalgal cover, seasonal trends in macroalgal abundance are an important consideration in the development of a long-term macroalgal monitoring protocol. Figure 9 shows the trends in mean *Ulva* cover in the 2008-2010 and 2013 datasets. The mean *Ulva* cover for all four of the 2013 sites combined does not exhibit the seasonal variation that is evident in the 2008-2010 dataset due to the extensive *Ulva* cover in September and November of 2008 and 2009. The highest mean *Ulva* cover observed between 2008 and 2010 was 38% averaged over the five sites in November of 2008 (Nettleton et al., 2011). In 2013, mean *Ulva* cover was actually highest in May, at 5%.

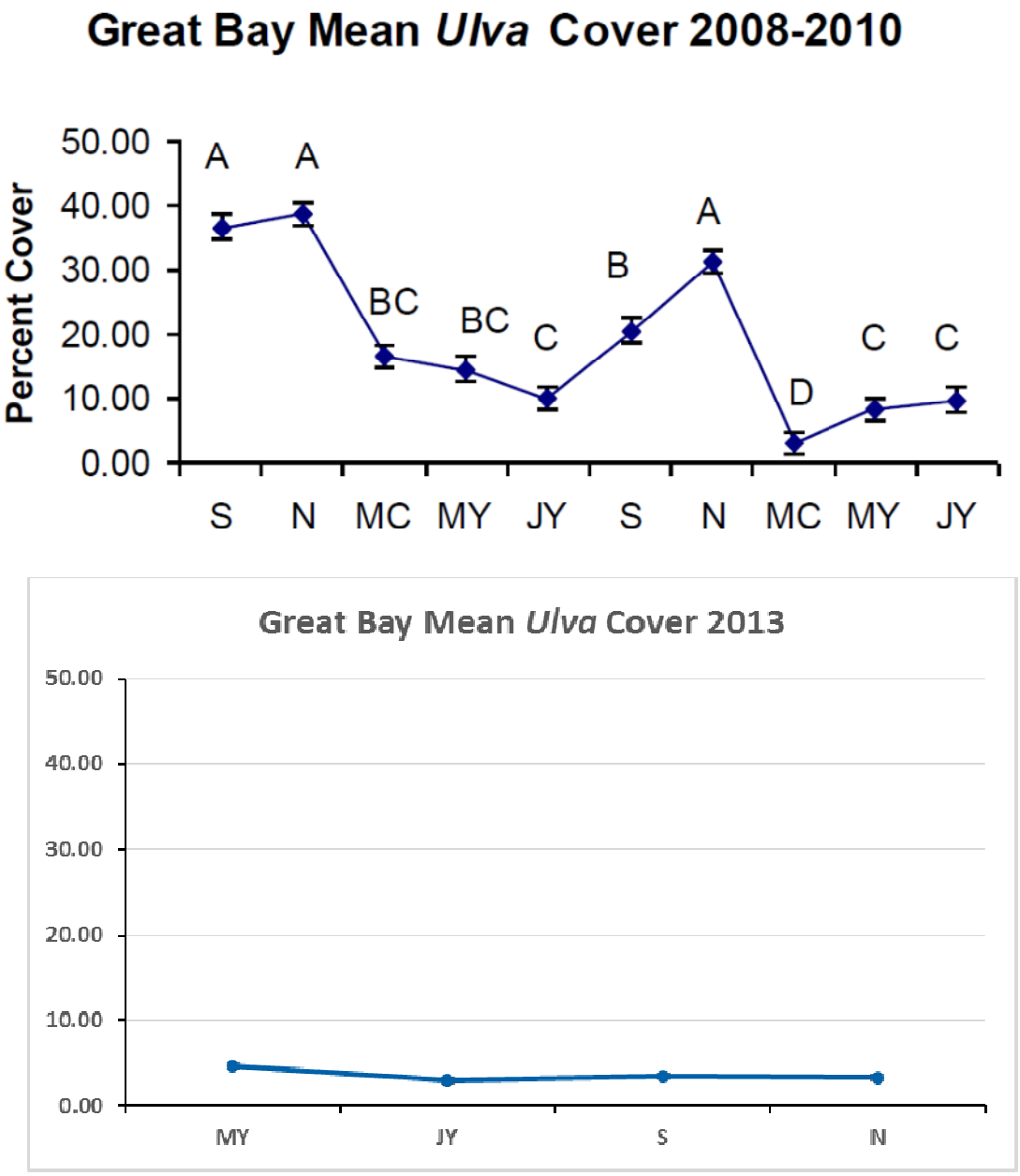
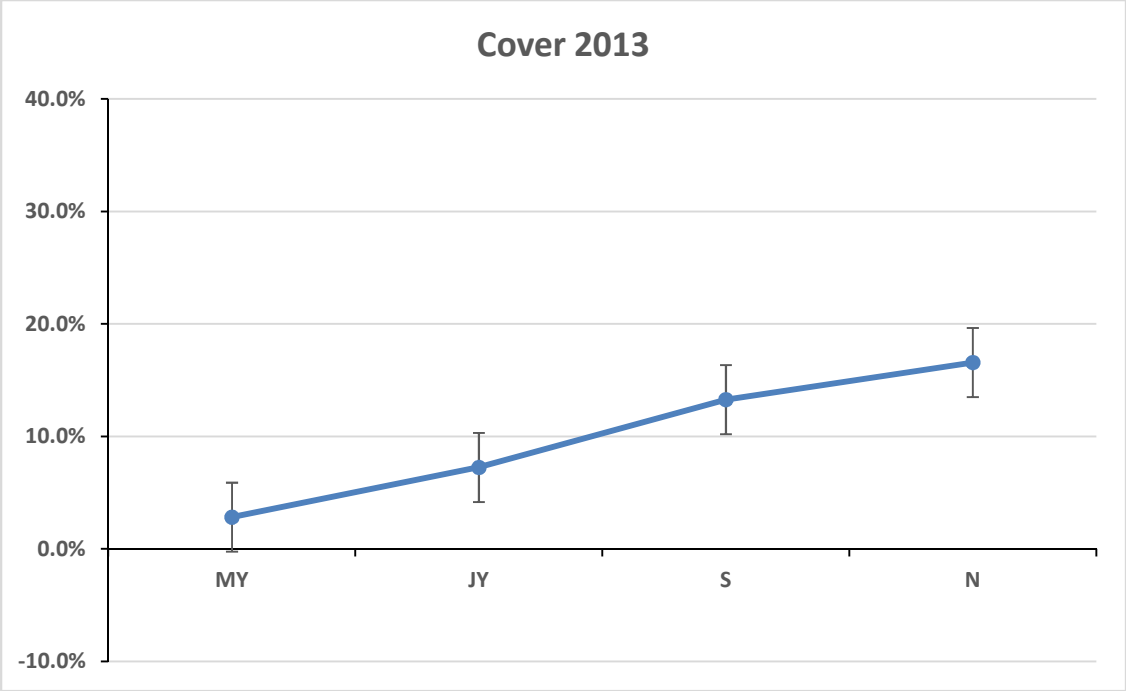


Figure 9. Monthly mean percent cover of *Ulva* in 0.25 m^2 quadrats at all sites included in the 2008-2010 and 2013 macroalgae transect sampling. The 2008-2010 figure was first published by Nettleton et al. (2011).



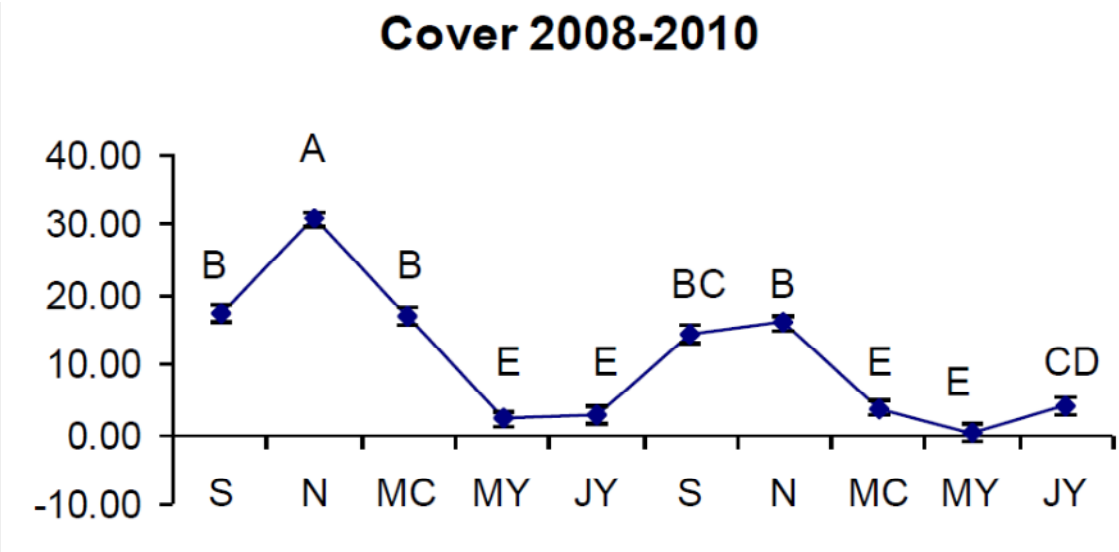


Figure 10. Monthly mean percent cover of *Gracilaria* in 0.25 m^2 quadrats at Depot Road, Lubberland Creek, and Sunset Farm (Southern Great Bay) in 2008-2010 and 2013. Sunset Farm was not sampled in 2013. The 2008-2010 figure was first published by Nettleton et al. (2011).

The data collected in 2013 also showed that *Gracilaria* dominated over *Ulva* after an initial spring dominance of *Ulva* at the sites in southern Great Bay Estuary where algal blooms had previously occurred. The seasonal trend observed is consistent with observations reported by Palmisciano et al. (2013) for Narragansett Bay, Rhode Island, where chlorophyte blooms,

defined as 71-100% cover of a 0.15 km^2 area, were observed most frequently in June and July of 2008, while rhodophyte blooms were observed more often in September of the same year. Future sampling will show whether or not the shift toward *Gracilaria* blooms since Nettleton's 2011 study is simply a one-year variation or a long-term trend.

2. Random Sampling

Our random sampling results are similar to those reported by Palmisciano et al. (2013) for the nearby Narragansett Bay Estuary between 2007 and 2011. They most often described the total macroalgal cover of Narragansett Bay as 11-40%, and most sections had 1-10% green algae and red algae cover and 0% brown algae cover (Palmisciano et al., 2013). A non-random ground-truthing assessment conducted in Narragansett Bay between 2007 and 2012 found the six most common species/genera of macroalgae at 37 sites were *Ulva*, *Agardhiella subulata* (C. Agardh) Kraft & M. J. Wynne, *Ceramium* Roth, *Polysiphonia* Greville/*Neosiphonia* M.-S. Kim and I.K. Lee, *Fucus*, and *Gracilaria tikvahiae* (Palmisciano et al., 2013). Based upon the random sampling, no taxa contested the prevalence of *Gracilaria* and *Ulva* in Great Bay Estuary. However, *Ceramium*, *Fucus*, and the *Polysiphonia* relative *Heterosiphonia* Montagne were among the less common macroalgae encountered.

Factors such as the presence of hard substrata or high wave energy significantly influence the likelihood of finding macroalgae at a sampling point. For example, the presence or absence of rocky intertidal habitat can change the cover of fucoid algae from nearly 100% to 0%. Although eight points had 90-100% macroalgal cover in the random sampling assessment, it was most common for a single type of macroalgae to occur in no more than 10% of a 0.25 m^2 plot (N = 32, which is half of the 64 points that contained any macroalgae).

Comparisons between the 2013 random sampling data and the aerial imagery analysis performed by Pe'eri et al. (2008), which estimated that 3% of the surface area of Great Bay was covered with macroalgae in August of 2007, are indirect. Because the average macroalgal cover in the area that we sampled in 2013 was roughly 12%, we estimated that macroalgae covered about 2.3% of the Estuary in August of 2013. Values of a random sampling procedure are its abilities to detect the movement of taxa to places where they have not been found previously and measure the integration of macroalgae in areas that are predominantly covered with other vegetation, such as rooted eelgrass or saltmarsh plants. Although our random sampling was able to account for small patches of macroalgae and estimates from aerial photographs have been reported to have lower accuracy when cover is less than 75% (Nezlin et al., 2007), macroalgal cover in August of 2013 appears to have been comparable to macroalgal cover in August of 2007.

Because the random point assessment surveyed all types of vegetation, it provides data that can be used to compare patterns of spatial variability in estuarine habitats. For instance, the 24 points that contained greater than 10% eelgrass cover had a mean percent cover value of 42.5 ± 30.1 SD. The mean percent macroalgal cover value of the 64 points that had macroalgae was 11.7 ± 24.8 SD, which shows that macroalgae were more spatially diffuse. Further insight into the competitive interactions between macroalgae and eelgrass may be possible by comparing the random sampling data against the eelgrass map Kappa Mapping, Inc. is producing for PREP with the aerial photographs it collected on August 24, 2013.

3. Isotope Ratio Analysis

Overall, low ^{15}N signatures were found in marine tissue samples and higher signatures in samples collected farther upstream in Great Bay Estuary. The stable isotope signatures of nitrogen in the algae showed general trends affected by nitrogen sources, but stable isotope analysis does not provide direct links to inputs. For example, samples from South Mill Pond in Portsmouth and Dover Point had somewhat higher ^{15}N values than would be expected based on their distance from the Atlantic coast. The ^{15}N enrichment observed in these samples suggests that the combined sewer overflows at South Mill Pond and the discharges from wastewater treatment plants near Dover Point are sources of elevated ^{15}N. In fact, the ^{15}N values for the samples collected from South Mill Pond and Dover Point were on par with those from the Oyster River near the Durham wastewater treatment plant. Because the Durham wastewater treatment plant is situated midway up a tidal river far from the Atlantic coast, the ^{15}N signature of the sample from the Oyster River does not show up as an anomalously high value. The ^{15}N signature of the sample collected near the Portsmouth wastewater treatment plant showed lower ^{15}N elevation than those collected near other wastewater discharges. It is likely that flushing from the Gulf of Maine moves and dilutes the nitrogen that is discharged from the Portsmouth wastewater treatment plant before local macroalgae are exposed to it.

At the upper end of the estuarine gradient, the *Ulva* collected from eelgrass beds had lower ^{15}N concentrations than the *Ulva* collected about the same distance upstream, but along the shoreline. Higher levels of biological activity in the shallow euphotic zone along the shoreline increase ^{15}N enrichment along the shoreline (Altabet et al., 1986). The elevated ^{15}N in the sample collected from the mudflats of Lubberland Creek Nature Preserve suggests the presence of a wildlife source of ^{15}N, such as waterfowl or a local septic source. Although it was collected from mudflats, the sample may have been exposed to elevated ^{15}N due to the reduction of nitrate by anaerobic bacteria in the surrounding salt marsh. It is also possible that ^{15}N reaches this site from wastewater discharges upstream. On the other hand, the *Ulva* collected at Lubberland Creek Nature Preserve may have originated several hundred meters away from the mudflats where it was found.

The significant trend in the ^{13}C signatures of these samples confirms the apparent spatial gradient found in ^{15}N signatures in *Ulva* tissue. All of the carbon isotope ratio data fell within the normal range of 27‰ and 5‰ (Michener and Lajtha, 2007). Carbon isotope ratios vary widely for macroalgae, because some macroalgae rely on benthic carbon stocks, which are typically enriched in ^{13}C (Michener and Lajtha, 2007), while others use the carbon stocks in suspended particulate matter and air. Furthermore, tissues of grasses that use C_4 carbon fixation, such as sugar, corn, and *Spartina* spp., show less ^{13}C depletion than the tissues of plants that use the more common C_3 carbon fixation path (Lajtha and Marshall, 1994). Therefore, ^{13}C signatures are high in organisms that use C_4 grasses as a carbon source. Decomposing human-derived waste products that are enriched in carbon derived from C_4 plants such as sugar and corn can provide such a carbon source for photosynthesizing macroalgae, especially further upstream within Great Bay Estuary. Decomposing saltmarsh grasses could also be a source of ^{13}C enrichment. The trend of increasing ^{13}C signatures one would expect to see along an estuarine gradient based on the relative availability of carbon from C_4 plants is evident in our results.

The ^{15}N concentrations in the *Ulva* collected in Great Bay Estuary were similar to the concentrations Raimon et al. (2013) observed in samples collected in spring from the Charente Estuary of France (6.2-10.1‰), but lower than the concentrations Pruell et al. (2006) observed in samples collected from Narragansett Bay, RI (9.44-12.42‰). Rather, they all fell within the range Titylanov et al. (2011) reported for *Ulva* sp. collected from southern Vietnam (5.0-9.9‰). Because the Charente Estuary is poorly mixed (Raimon et al., 2013), one would expect the enriched signatures of ^{15}N in the euphotic zone to be even greater than in well-mixed estuaries such as Great Bay Estuary. Pruell et al. (2006) found that an increase in ammonia in the water column was correlated with the high ^{15}N signatures they observed. The range of ^{15}N signatures across the studies cited, however, is relatively narrow. Such comparisons suggest that, at least during the sample collection period for this study, the influence of nitrogen from wastewater sources in Great Bay Estuary is comparable to eutrophied estuaries in other parts of the world. One appealing feature of isotope-based monitoring is the fact that isotope signatures can signal the presence of eutrophication before drastic and possibly irreversible shifts in ecological communities occur (Viana et al., 2011).

Recommendations

Of the three research approaches explored, we expect the transect sampling approach to be the most useful and practical means for obtaining the data that are needed to assess progress toward the PREP management goal of no increasing trends in macroalgae (PREP, 2013). In order for the transect sampling approach to be successful over the long-term, it is important to consider what practices can be implemented to ensure consistency in data collection and management from year to year, how data accuracy and representativeness will suit management goals, and how the selected monitoring approach fits into the wider universe of possible monitoring options.

Transect Monitoring

Planned monitoring for the 2014 field season will add four new sites and retain one of the sites monitored in 2013 in the pool of transect sampling sites to create a rotation of eight transect sampling sites. As Nettleton et al. (2011) observed and our 2013 results confirmed, the protocol should include sampling sites distributed around Great Bay Estuary. It is particularly important to include sites from different parts of the Estuary in the same monitoring rotation because some parts of the Estuary are more conducive to accumulating macroalgal blooms than others, as described in the discussion of blooms in southern Great Bay Estuary.

Percent cover estimates should be collected in July, August, and September. One can expect to capture peak total macroalgal cover and biomass values by sampling in August or September, before the onset of senescence in eelgrass and saltmarsh grasses frees macroalgal mats to migrate. Raposa et al. (2011) found that basing the number of sites they sampled on the variability in macroalgal cover in July-August and September also sufficiently captured the variability in macroalgal cover between tidal zones. Using a power analysis (Snedecor and Cochran, 1980), Raposa et al. (2011) were able to determine that based on the coefficient of variation of their macroalgal cover estimates and a desired confidence interval of 20%, they needed to estimate macroalgal cover at 30 sites around Narragansett Bay. The coefficient of variation of their estimates was higher in June and October, thus necessitating more samples to achieve the same level of precision. To achieve adequate precision from fewer samples is also desirable for monitoring in Great Bay Estuary, and our results did not show that sampling before July or after September was important to characterize total macroalgal cover.

For field sampling on the ground, the two greatest potential sources of measurement error when returning to a site to resample in a different year are: 1) locating the same sampling space and, 2) consistently estimating percent cover. We installed sampling markers and stored the spatial coordinates of the markers in a GPS unit, as documented in APPENDIX A. As previously stated earlier, previous studies of macroalgae in Great Bay Estuary have used a point-intercept approach to estimate percent cover. The *Ulva* cover at Lubberland Creek Nature Preserve shows that the presence/absence data generated by point-intercept sampling across a quadrat may not scale-up to percent cover estimates for the quadrat. However, estimating percent cover for an entire quadrat is more subjective than aggregating point-intercept data. Because it is typically necessary to have multiple individuals estimating percent cover in the field and to replace individuals working on a project over time, it is ideal to establish a set of photographs of plots to present to all individuals to estimate percent cover so that variability in the data due to

differences in observer perceptions can be minimized. Photographs from the May, July, and November sampling in Great Bay Estuary in 2013 have been compiled on an external hard drive housed with PREP. Photographs are not available from September because of damage to the camera that occurred during sampling. Copies of the photographs are available as electronic files by request.

Macroalgal biomass is an important parameter to track because of its compatibility with policies and regulations that are based on nitrogen concentrations. Under the 2014 Great Bay Estuary macroalgal monitoring protocol, biomass samples will be collected during one of the sampling events. Biomass sampling is time-consuming and more expensive than estimating percent cover, but the level of precision achieved in the linear models that predict biomass from cover may be insufficient for long-term management. Non-random sampling may be needed to develop models for certain species. It is difficult to collect enough data using a non-biased sampling approach for species or morphological "forms" that occur infrequently or have extremely high or low biomass. For example, the protocol requirement that all material attached within the quadrat area be counted as cover in the quadrat resulted in a wide range of masses for high cover values of *Ascophyllum nodosum*. Another potential way to improve biomass estimates from regressions is to measure the thickness of the algal mat. Possible site-specific differences in the relationship between biomass and percent cover (Rollon et al., 2003) further advocate the need for additional biomass data collection in the Estuary.

The macroalgal monitoring effort for Great Bay Estuary for 2014 will need to make trade-offs between the quality of the data collected and the cost of collecting the data. We estimate that each site visit will require 1-2 hours of fieldwork, which is more time-consuming than would be necessary to perform a rapid assessment. For example, Raposa et al. (2011) were able to estimate percent cover, without species composition, twice at each of 31 sites in 24 hours of fieldwork spread across 6 days. On the other hand, an important aspect of collecting field data on the ground is to take the time to capture site variability as well as species and genus-level data. Although it would be ideal to develop a monitoring protocol that would also allow PREP to track changes in the distribution and abundance of the non-native invasive *Gracilaria vermiculophylla*, the DNA sequencing that is required to make a true distinction is cost-prohibitive. At this time, the effort and cost of recognizing the genus *Gracilaria* to assess its absolute and relative abundance is worthwhile, but not to the species level.

Unexplored Monitoring Options

Aerial photography could prove to be an efficient way to collect data across the Estuary during peak biomass. Palmisciano et al. (2013) recommend using a GIS file that categorizes the availability of substrata and shoreline geometry as predictors of macroalgal habitat to select a diverse range of points to check macroalgal cover on the ground. Designing the groundtruthing process in this way would help address the challenges encountered by attempting to randomly sample 50 points of each of four cover types. Palmisciano et al. (2013) also recommend using standardized field data sheets and collecting biomass data in the field and using vertical aerial photography (so that scaling is consistent across photographs), a standard photograph area, and the ImageJ software tool for interpretation. To fully utilize remote sensing to monitor macroalgae, one would need field observations from the ground to confirm the species/genera that are found in the remote sensing results as well as the thickness of macroalgal mats.

Tidal rivers may be a sampling area of interest in the future, because they can be used to isolate nutrient sources within specific watersheds that drain into Great Bay Estuary. Field observations (Cianciola, 2014) suggest that assigning tidal rivers to a separate sample frame could allow future researchers to collect representative random samples from these areas. The Salmon and Cocheco Rivers are shallow enough to support *Ulva*, and the Oyster River supports *Gracilaria* and *Ulva* populations. Few random sampling points fell in tidal rivers because of their small area relative to the Bay, and even fewer actually captured the macroalgae that were present.

Macroalgal monitoring in Great Bay Estuary could incorporate the assessment of preserved specimens. A collection such as the one Mathieson and Hehre (1986) established in the University of New Hampshire Hodgdon Herbarium could be analyzed to describe environmental conditions such as nitrogen availability in retrospect. For example, Viana et al. (2011) used specimens preserved in an environmental specimen bank to detect trends of decreasing ^{15}N over time in three species of *Fucus*. Viana et al. interpret their results as a reflection of the success of new regulations requiring sewage treatment for populations larger than 2,000 people.

Management

A primary objective of a long-term monitoring program is that management actions may be taken based on the data collected. Even though a preliminary assessment of macroalgal change in the Estuary is presented in this report, it will likely require several (3-5) years of monitoring to produce sufficient data to describe any statistically significant trends in macroalgal abundance as an indicator of estuarine health. Management actions may include activities such as implementing best management practices in the watershed to control nitrogen inputs or efforts to control the spread of invasive species. For example, although it does not specify what actions must be taken, the European Commission Water Framework Directive requires that water quality be maintained such that the observed taxa of macroalgae, seagrasses, saltmarsh plants, and phytoplankton are consistent with undisturbed conditions and that anthropogenic activities do not cause detectable changes in macroalgal abundance (Wilkinson et al., 2007).

Studies from other estuaries have shown that once a nitrogen management plan is in place, usually in the form of a total maximum daily load (TMDL) and/or nitrogen credit trading program, it takes a few years for the ecosystem to fully respond to the change in nitrogen availability. Palmisciano et al. (2013) encouraged consideration of management options that help engage the community and facilitate recovery until the desired effects of a nitrogen management plan are manifested, such as collecting macroalgae from coastal property owners as yard waste. The collected macroalgae could then be processed and applied as a fertilizer under appropriate circumstances. Important benchmarks along the road to recovery include first the reduction of macroalgae populations and then the rebound of eelgrass populations. Eelgrass populations thrive when algal blooms are controlled, because reducing macroalgal mats allows more light to reach rooted aquatic vegetation (Beem and Short, 2009). Recovery in an estuary of Long Island Sound showed a two-year time lag before *Ulva* populations returned to baseline levels in Mumford Cove, CT (Vaudrey et al., 2002). Eelgrass returned to the Cove 10 years after a nitrogen management plan was first implemented and the population took another 5 years to stabilize (Vaudrey et al., 2002). The recovery period observed in Long Island Sound is comparable to the time lag of 1-3 years before macroalgae populations responded to decreased nitrogen loading in Tampa Bay, Florida (Johansson, 2002) and the 8-10 years required to recover

submerged aquatic vegetation as reported for Chesapeake Bay (Maryland Department of Natural Resources, 2002). The competitive interactions between macroalgae and eelgrass in Great Bay Estuary may still be weak enough now that the time-to-recovery following the implementation of a nitrogen management plan would be on the shorter end of the spectrum observed in other systems.

Public education is a critical component of most natural resource management plans. Our study provided an opportunity for volunteers to have a positive, interdisciplinary educational experience at a local level. There is potential to expand that experience across a national level. The time volunteers spent learning to implement the monitoring protocol gave them first-hand insight into how their individual and community actions can support the natural resources they value, such as resources within or associated with Great Bay Estuary. At the national level, the Great Bay Estuary macroalgal monitoring protocol will also be available to other National Estuary Programs and National Estuarine Research Reserves to coordinate monitoring programs across the country and thus build a more robust dataset that allows both for comparison between regions and the capability to make statements about the ecological condition of individual estuarine systems.

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APPENDIX A. Geospatial coordinates of points on 2013 macroalgal sampling transects.

Site	Elevation (meters from MLLW)	Latitude	Longitude
Cedar Point	-0.8	43.128728	-70.852965
Cedar Point	-0.8	43.128685	-70.852555
Cedar Point	-0.8	43.128694	-70.852388
Cedar Point	0.0	43.129245	-70.852823
Cedar Point	0.0	43.129308	-70.852656
Cedar Point	0.0	43.129160	-70.853258
Cedar Point	0.5	43.129299	-70.853273
Cedar Point	0.5	43.129379	-70.852714
Cedar Point	0.5	43.129335	-70.852833
Cedar Point	1.0	43.129384	-70.852866
Cedar Point	1.0	43.129407	-70.852726
Cedar Point	1.0	43.129387	-70.853276
Cedar Point	1.5	43.129419	-70.852872
Cedar Point	1.5	43.129409	-70.852720
Depot Road	0.5	43.056188	-70.896607
Depot Road	0.5	43.056234	-70.896761
Depot Road	0.5	43.056344	-70.897168
Depot Road	1.0	43.056112	-70.896667
Depot Road	1.0	43.056109	-70.896816
Depot Road	1.0	43.056233	-70.897204
Depot Road	1.5	43.056087	-70.896680
Depot Road	1.5	43.056096	-70.896809
Depot Road	1.5	43.056221	-70.897205
Lubberland Creek	0.5	43.073519	-70.902854
Lubberland Creek	0.5	43.074162	-70.902316
Lubberland Creek	0.5	43.073872	-70.902575
Lubberland Creek	1.0	43.074474	-70.903233
Lubberland Creek	1.0	43.074267	-70.903394
Lubberland Creek	1.0	43.073968	-70.903693
Lubberland Creek	1.5	43.074481	-70.903287
Lubberland Creek	1.5	43.074303	-70.903463
Lubberland Creek	1.5	43.073995	-70.903754

Site	Elevation (meters from MLLW)	Latitude	Longitude
Wagon Hill Farm	0.0	43.124094	-70.872409
Wagon Hill Farm	0.0	43.124111	-70.872218
Wagon Hill Farm	0.0	43.124058	-70.872747
Wagon Hill Farm	0.5	43.124541	-70.872949
Wagon Hill Farm	0.5	43.124572	-70.872601
Wagon Hill Farm	0.5	43.124699	-70.872493
Wagon Hill Farm	1.0	43.124571	-70.872977
Wagon Hill Farm	1.0	43.124653	-70.872643
Wagon Hill Farm	1.0	43.124741	-70.872497
Wagon Hill Farm	1.5	43.124617	-70.872980
Wagon Hill Farm	1.5	43.124728	-70.872661

APPENDIX B. . Mean monthly percent cover values for samples of 0.25 m^2 quadrats.

Table B-1. Cedar Point

	Ulva (foliose)			*Gracilaria*			*Ascophyllum nodosum*			*Fucus vesiculosus*		
MY	0.45%	±	0.52%	0.45%	±	1.51%	0.00%	±	0.00%	12.82%	±	19.98%
JY	1.86%	±	5.25%	0.50%	±	1.34%	2.86%	±	10.69%	9.71%	±	17.44%
S	1.64%	±	5.30%	0.43%	±	1.34%	0.00%	±	0.00%	3.29%	±	7.21%
N	1.57%	±	5.32%	1.86%	±	5.39%	0.07%	±	0.27%	4.29%	±	10.89%

	Ulva (tubular)			*Ceramium virgatum*			*Cladophora sericea*			*Chondrus crispus*		
MY	0.00%	±	0.00%	0.00%	±	0.00%	2.91%	±	6.39%	0.00%	±	0.00%
JY	1.43%	±	3.06%	0.00%	±	0.00%	6.79%	±	21.27%	1.43%	±	5.35%
S	0.14%	±	0.36%	6.43%	±	17.37%	7.50%	±	18.48%	0.00%	±	0.00%
N	1.79%	±	3.17%	7.14%	±	19.78%	7.50%	±	14.24%	0.00%	±	0.00%

	Heterosiphonia japonica			*Polysiphonia stricta*			*Pylliella littoralis*			*Spartina alterniflora*		
MY	0.00%	±	0.00%	0.00%	±	0.00%	0.27%	±	0.47%	4.27%	±	6.53%
JY	0.00%	±	0.00%	0.00%	±	0.00%	0.00%	±	0.00%	8.29%	±	19.54%
S	1.86%	±	3.70%	0.00%	±	0.00%	0.00%	±	0.00%	6.79%	±	16.36%
N	2.50%	±	8.03%	0.00%	±	0.00%	0.00%	±	0.00%	2.29%	±	5.74%

	Spartina patens			*Vaucheria*		
MY	0.00%	±	0.00%	0.00%	±	0.00%
JY	0.00%	±	0.00%	0.71%	±	2.67%
S	0.00%	±	0.00%	8.57%	±	16.10%
N	0.00%	±	0.00%	0.86%	±	2.66%

Table B-2. Depot Road.

	Ulva (foliose)			*Gracilaria*			*Ascophyllum nodosum*			*Fucus vesiculosus*		
MY	16.67%	±	20.46%	10.22%	±	16.25%	7.78%	±	23.33%	2.78%	±	3.63%
JY	6.22%	±	6.40%	28.11%	±	36.38%	0.00%	±	0.00%	4.11%	±	6.85%
S	5.11%	±	6.99%	42.78%	±	45.15%	0.56%	±	1.67%	3.56%	±	6.97%
N	2.44%	±	3.50%	43.00%	±	47.42%	0.00%	±	0.00%	9.22%	±	20.12%

	Ulva (tubular)			*Ceramium virgatum*			*Cladophora sericea*			*Chondrus crispus*		
MY	0.00%	±	0.00%	0.00%	±	0.00%	6.67%	±	10.90%	0.00%	±	0.00%
JY	1.22%	±	2.17%	0.33%	±	0.50%	0.56%	±	1.67%	0.00%	±	0.00%
S	0.44%	±	0.53%	0.00%	±	0.00%	0.00%	±	0.00%	0.00%	±	0.00%
N	0.44%	±	0.53%	0.56%	±	1.67%	3.33%	±	5.00%	0.00%	±	0.00%

	Heterosiphonia japonica			*Polysiphonia stricta*			*Pylliella littoralis*			*Spartina alterniflora*		
MY	0.00%	±	0.00%	0.00%	±	0.00%	1.11%	±	3.33%	16.11%	±	24.21%
JY	0.00%	±	0.00%	0.00%	±	0.00%	0.00%	±	0.00%	21.67%	±	31.42%
S	0.00%	±	0.00%	0.00%	±	0.00%	0.00%	±	0.00%	20.56%	±	30.05%
N	0.00%	±	0.00%	0.00%	±	0.00%	1.22%	±	2.17%	12.89%	±	25.08%

	Spartina patens			*Vaucheria*		
MY	0.00%	±	0.00%	0.00%	±	0.00%
JY	0.00%	±	0.00%	0.00%	±	0.00%
S	0.00%	±	0.00%	0.00%	±	0.00%
N	0.00%	±	0.00%	0.00%	±	0.00%

Table B-3. Lubberland Creek.

	Ulva (foliose)			*Gracilaria*			*Ascophyllum nodosum*			*Fucus vesiculosus*		
MY	3.00%	±	4.27%	1.78%	±	2.44%	0.00%	±	0.00%	1.11%	±	2.20%
JY	2.67%	±	4.18%	3.33%	±	6.61%	0.00%	±	0.00%	2.56%	±	6.56%
S	7.78%	±	13.94%	15.56%	±	24.55%	0.00%	±	0.00%	2.44%	±	6.60%
N	5.56%	±	8.82%	27.78%	±	41.77%	0.00%	±	0.00%	5.56%	±	13.33%

	Ulva (tubular)			*Ceramium virgatum*			*Cladophora sericea*			*Chondrus crispus*		
MY	0.00%	±	0.00%	0.00%	±	0.00%	2.22%	±	4.41%	0.00%	±	0.00%
JY	0.00%	±	0.00%	0.00%	±	0.00%	0.00%	±	0.00%	0.00%	±	0.00%
S	0.22%	±	0.44%	0.00%	±	0.00%	0.11%	±	0.33%	0.00%	±	0.00%
N	0.78%	±	1.64%	0.00%	±	0.00%	0.00%	±	0.00%	0.00%	±	0.00%

	Heterosiphonia japonica			*Polysiphonia stricta*			*Pylliella littoralis*			*Spartina alterniflora*		
MY	0.00%	±	0.00%	0.00%	±	0.00%	0.00%	±	0.00%	45.56%	±	36.44%
JY	0.00%	±	0.00%	0.00%	±	0.00%	0.00%	±	0.00%	53.33%	±	41.83%
S	0.11%	±	0.33%	0.11%	±	0.33%	0.00%	±	0.00%	46.67%	±	37.08%
N	0.11%	±	0.33%	0.00%	±	0.00%	0.00%	±	0.00%	41.11%	±	36.55%

	Spartina patens			*Vaucheria*		
MY	0.00%	±	0.00%	0.67%	±	1.66%
JY	0.00%	±	0.00%	0.00%	±	0.00%
S	0.00%	±	0.00%	1.78%	±	3.49%
N	0.00%	±	0.00%	0.00%	±	0.00%

Table B-4. Wagon Hill Farm.

	Ulva (foliose)			*Gracilaria*			*Ascophyllum nodosum*			*Fucus vesiculosus*		
MY	0.27%	±	0.47%	0.00%	±	0.00%	0.09%	±	0.30%	0.91%	±	2.02%
JY	0.00%	±	0.00%	0.00%	±	0.00%	0.45%	±	1.51%	2.73%	±	6.07%
S	0.00%	±	0.00%	0.00%	±	0.00%	0.09%	±	0.30%	0.18%	±	0.40%
N	0.00%	±	0.00%	0.00%	±	0.00%	0.45%	±	1.51%	0.09%	±	0.30%

	Ulva (tubular)			*Ceramium virgatum*			*Cladophora sericea*			*Chondrus crispus*		
MY	0.00%	±	0.00%	0.00%	±	0.00%	2.36%	±	4.06%	0.00%	±	0.00%
JY	0.27%	±	0.47%	0.00%	±	0.00%	0.00%	±	0.00%	0.00%	±	0.00%
S	0.91%	±	3.02%	0.00%	±	0.00%	5.55%	±	18.06%	0.00%	±	0.00%
N	1.82%	±	4.05%	0.00%	±	0.00%	6.36%	±	21.11%	0.00%	±	0.00%

	Heterosiphonia japonica			*Polysiphonia stricta*			*Pylliella littoralis*			*Spartina alterniflora*		
MY	0.00%	±	0.00%	0.09%	±	0.30%	0.18%	±	0.40%	8.27%	±	23.98%
JY	0.00%	±	0.00%	0.00%	±	0.00%	0.00%	±	0.00%	7.73%	±	24.02%
S	1.00%	±	3.00%	0.00%	±	0.00%	0.00%	±	0.00%	6.82%	±	21.01%
N	0.91%	±	3.02%	0.00%	±	0.00%	3.82%	±	8.01%	5.55%	±	18.06%

	Spartina patens			*Vaucheria*		
MY	6.36%	±	21.11%	0.00%	±	0.00%
JY	7.27%	±	24.12%	1.45%	±	3.21%
S	6.36%	±	21.11%	1.00%	±	3.00%
N	2.73%	±	6.47%	4.55%	±	9.34%

APPENDIX C. Estimated mean monthly biomass (g) for samples of 0.25 m^2 quadrats.

Table C-1. Cedar Point.

	Ulva (foliose)			*Gracilaria*			*Ascophyllum nodosum*			*Fucus vesiculosus*		
MY	0.00	±	0.00	0.2	±	0.5	0.0	±	0.0	31.6	±	46.7
JY	0.00	±	0.00	0.2	±	0.5	9.8	±	32.5	27.7	±	43.0
S	0.00	±	0.00	0.2	±	0.5	0.0	±	0.0	9.2	±	18.1
N	0.00	±	0.00	0.8	±	2.1	0.0	±	0.0	12.3	±	27.4

	Ulva (tubular)			*Ceramium virgatum*			*Cladophora sericea*			*Chondrus crispus*		
MY	0.0	±	0.0	0.0	±	0.0	1.2	±	2.6	0.0	±	0.0
JY	0.5	±	0.9	0.0	±	0.0	3.3	±	9.2	0.0	±	0.0
S	0.0	±	0.0	0.0	±	0.0	3.7	±	7.9	0.0	±	0.0
N	0.6	±	0.9	0.0	±	0.0	3.7	±	6.0	0.0	±	0.0

	Heterosiphonia japonica			*Polysiphonia stricta*			*Pylliella littoralis*			*Spartina alterniflora*		
MY	0.0	±	0.0	0.0	±	0.0	0.0	±	0.0	4.1	±	6.7
JY	0.0	±	0.0	0.0	±	0.0	0.0	±	0.0	10.6	±	22.0
S	0.1	±	0.4	0.0	±	0.0	0.0	±	0.0	8.7	±	18.4
N	0.0	±	0.0	0.0	±	0.0	0.0	±	0.0	2.8	±	6.5

Table C-2. Depot Road.

	Ulva (foliose)			*Gracilaria*			*Ascophyllum nodosum*			*Fucus vesiculosus*		
MY	4.4	±	5.4	4.5	±	6.2	20.9	±	62.8	6.3	±	8.2
JY	1.6	±	1.7	9.8	±	12.9	0.0	±	0.0	9.3	±	15.5
S	1.3	±	1.8	15.0	±	15.9	1.5	±	4.5	7.5	±	16.0
N	0.6	±	0.9	15.0	±	16.7	0.0	±	0.0	20.1	±	45.8

	Ulva (tubular)			*Ceramium virgatum*			*Cladophora sericea*			*Chondrus crispus*		
MY	0.0	±	0.0	0.0	±	0.0	2.6	±	4.2	0.0	±	0.00
JY	0.3	±	0.6	0.0	±	0.0	0.2	±	0.6	0.0	±	0.00
S	0.0	±	0.0	0.0	±	0.0	0.0	±	0.0	0.0	±	0.00
N	0.0	±	0.0	0.3	±	0.9	1.3	±	1.9	0.0	±	0.00

	Heterosiphonia japonica			*Polysiphonia stricta*			*Pylliella littoralis*			*Spartina alterniflora*		
MY	0.00	±	0.00	0.00	±	0.00	0.3	±	1.0	16.3	±	24.5
JY	0.00	±	0.00	0.00	±	0.00	0.0	±	0.0	21.9	±	31.8
S	0.00	±	0.00	0.00	±	0.00	0.0	±	0.0	20.8	±	30.4
N	0.00	±	0.00	0.00	±	0.00	0.3	±	0.6	12.9	±	25.4

Table C-3. Lubberland Creek.

	Ulva (foliose)			*Gracilaria*			*Ascophyllum nodosum*			*Fucus vesiculosus*		
MY	0.7	±	1.2	0.6	±	0.9	0.0	±	0.0	2.5	±	5.0
JY	0.6	±	1.2	1.2	±	2.3	0.0	±	0.0	5.5	±	14.9
S	2.0	±	3.6	5.5	±	8.6	0.0	±	0.0	5.0	±	15.1
N	1.5	±	2.3	9.8	±	14.7	0.0	±	0.0	12.6	±	30.1

	Ulva (tubular)			*Ceramium virgatum*			*Cladophora sericea*			*Chondrus crispus*		
MY	0.0	±	0.0	0.0	±	0.0	0.9	±	1.7	0.0	±	0.00
JY	0.0	±	0.0	0.0	±	0.0	0.0	±	0.0	0.0	±	0.00
S	0.0	±	0.0	0.0	±	0.0	0.0	±	0.0	0.0	±	0.00
N	0.1	±	0.4	0.0	±	0.0	0.0	±	0.0	0.0	±	0.00

	Heterosiphonia japonica			*Polysiphonia stricta*			*Pylliella littoralis*			*Spartina alterniflora*		
MY	0.00	±	0.00	0.00	±	0.00	0.0	±	0.0	46.1	±	36.9
JY	0.00	±	0.00	0.00	±	0.00	0.0	±	0.0	54.0	±	42.3
S	0.00	±	0.00	0.00	±	0.00	0.0	±	0.0	47.2	±	37.5
N	0.00	±	0.00	0.00	±	0.00	0.0	±	0.0	41.6	±	37.0

Table C-4. Wagon Hill Farm.

	Ulva (foliose)			*Gracilaria*			*Ascophyllum nodosum*			*Fucus vesiculosus*		
MY	0.00	±	0.00	0.00	±	0.00	0.0	±	0.0	2.1	±	4.6
JY	0.00	±	0.00	0.00	±	0.00	1.2	±	4.1	6.2	±	13.7
S	0.00	±	0.00	0.00	±	0.00	0.2	±	0.8	0.0	±	0.0
N	0.00	±	0.00	0.00	±	0.00	1.2	±	4.1	0.0	±	0.0

	Ulva (tubular)			*Ceramium virgatum*			*Cladophora sericea*			*Chondrus crispus*		
MY	0.0	±	0.0	0.0	±	0.0	0.9	±	1.6	0.0	±	0.00
JY	0.0	±	0.0	0.0	±	0.0	0.0	±	0.0	0.0	±	0.00
S	0.2	±	0.8	0.0	±	0.0	2.1	±	7.0	0.0	±	0.00
N	0.5	±	1.1	1.1	±	2.4	2.5	±	8.2	0.0	±	0.00

	Heterosiphonia japonica			*Polysiphonia stricta*			*Pylliella littoralis*			*Spartina alterniflora*		
MY	0.00	±	0.00	0.00	±	0.00	0.0	±	0.0	8.3	±	24.3
JY	0.00	±	0.00	0.00	±	0.00	0.0	±	0.0	7.8	±	24.3
S	0.00	±	0.00	0.00	±	0.00	0.3	±	0.9	6.9	±	21.3
N	0.00	±	0.00	0.00	±	0.00	0.3	±	0.9	5.5	±	18.3

APPENDIX D. Linear models that predict macroalgal biomass from ground cover.

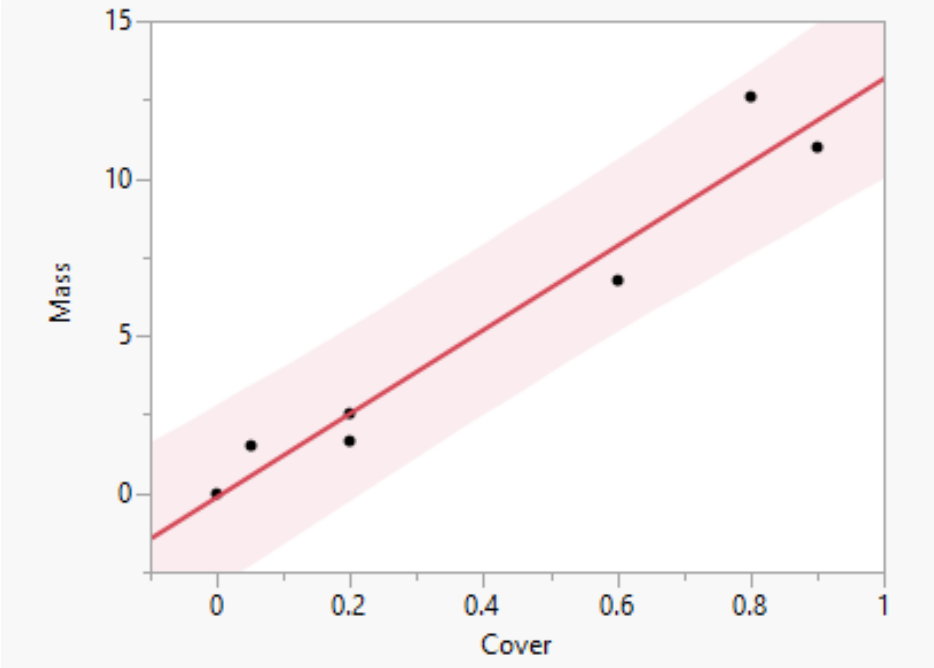

Figure D-1. Model of *Ceramium virgatum* biomass predicted from ground cover. Shading around line of best fit encompasses 90% confidence interval for individual predictions.

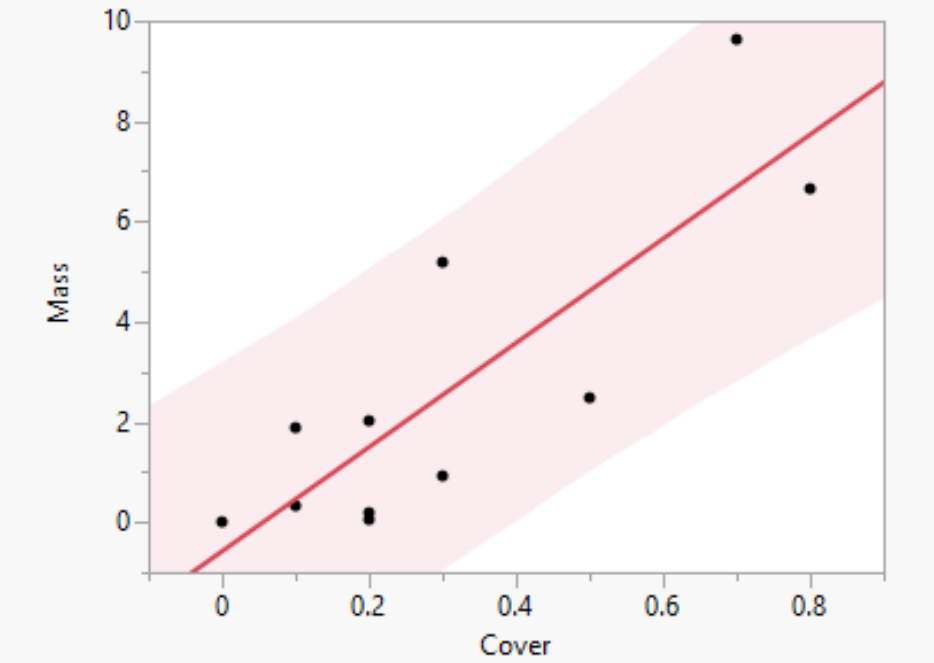

Figure D-11. Model of *Cladophora sericea* biomass predicted from ground cover. Shading around line of best fit encompasses 90% confidence interval for individual predictions.

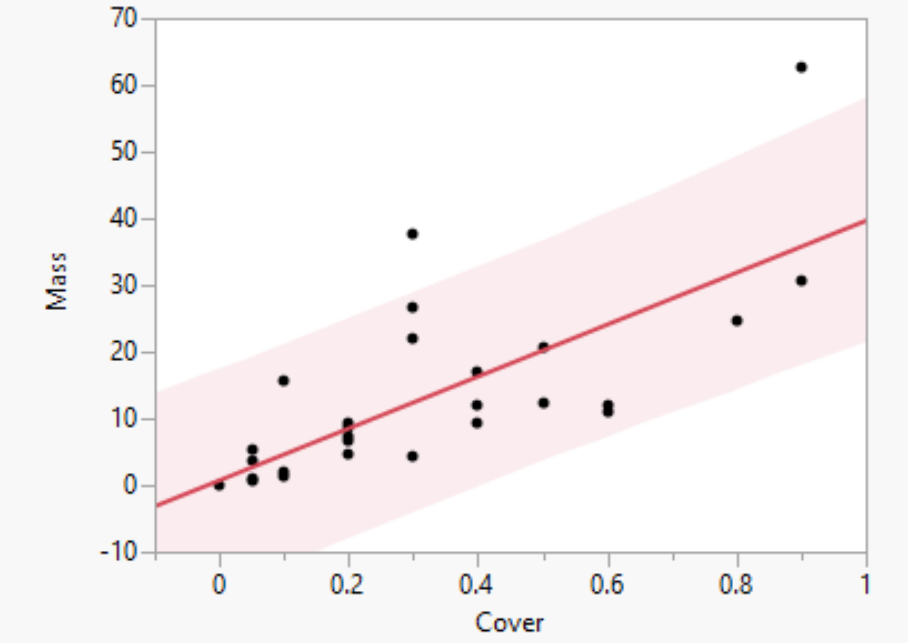

Figure D-12. Model of *Fucus vesiculosus* biomass predicted from ground cover. Shading around line of best fit encompasses 90% confidence interval for individual predictions.

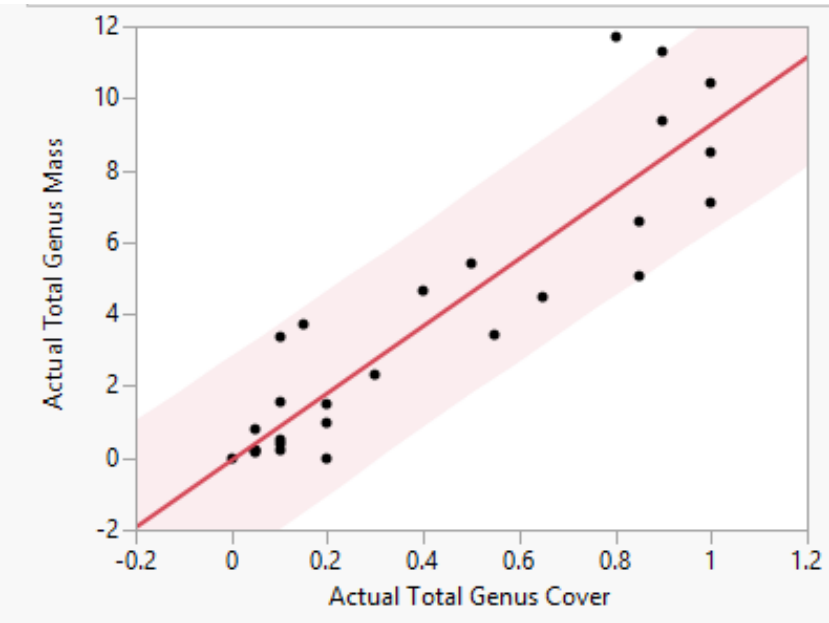

Figure D-13. Model of *Gracilaria* biomass predicted from ground cover. Shading around line of best fit encompasses 90% confidence interval for individual predictions.

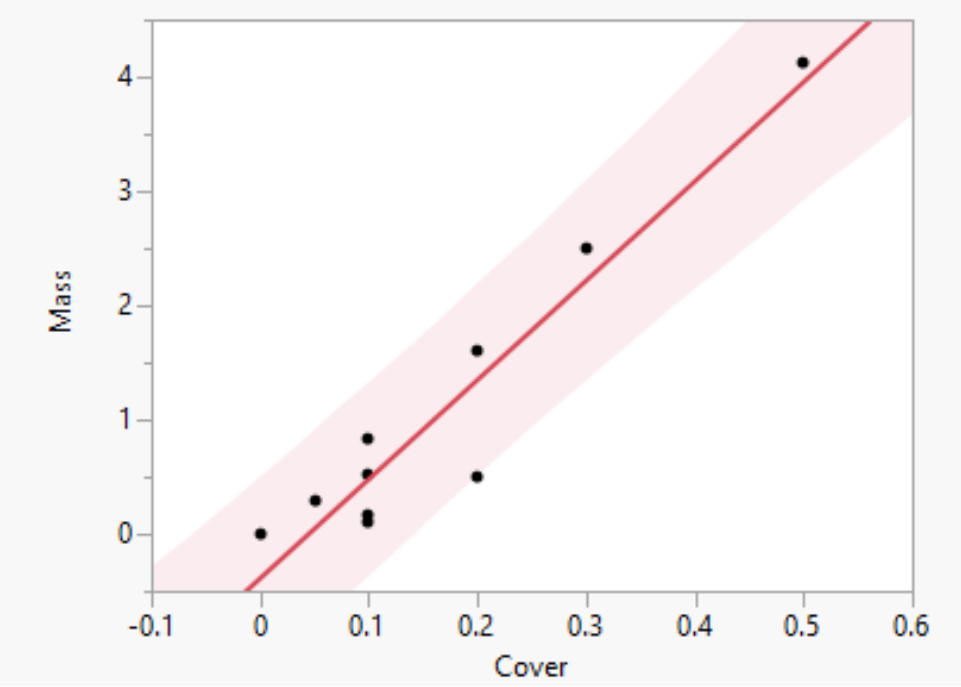

Figure D-14. Model of *Pyliella littoralis* biomass predicted from ground cover. Shading around line of best fit encompasses 90% confidence interval for individual predictions.

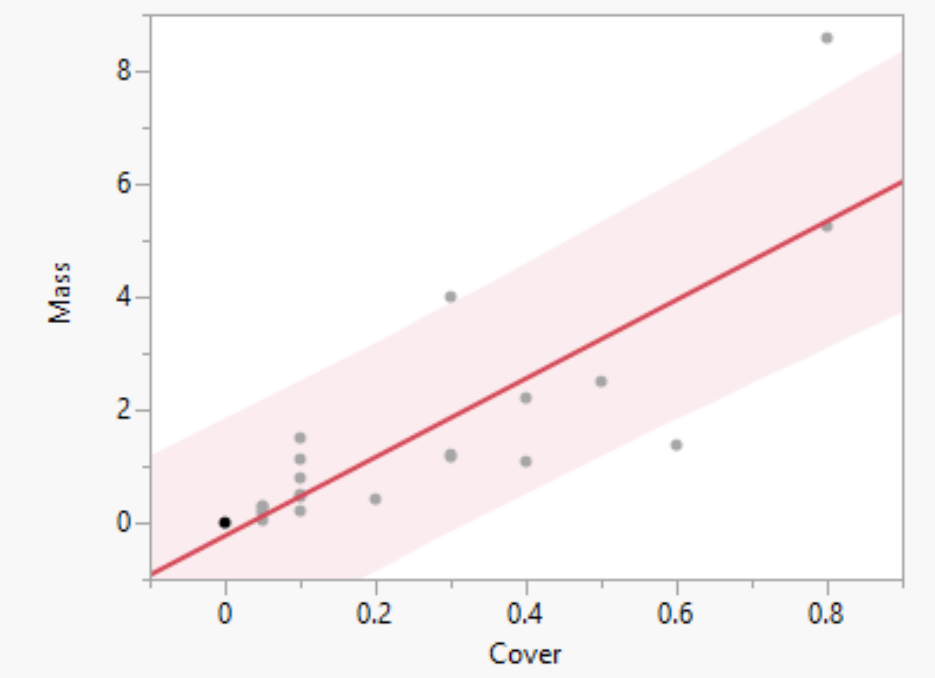

Figure D-15. Model of foliose *Ulva* biomass predicted from ground cover. Shading around line of best fit encompasses 90% confidence interval for individual predictions.

APPENDIX E. Standardized data sheet for estimating macroalgal cover at random sampling sites.

2013 Macroalgal Survey Instructions:

1. Deploy frame with sink and buoy attached when within 20 feet of point.
2. If you can touch ground, reposition attached vegetation so that everything falls on the same side of the frame as its roots/thalli.
3. If you have a camera with you, take a picture of the frame.
4. Have person holding frame estimate percentage of area inside frame covered by each species to nearest multiple of 10 (or <10) for second person to record below. Do not dig under top layer of ground cover and do not count dead material. Collect a piece of any alga that cannot be identified and bring to JEL in a bag labeled with point number & coordinates ASAP.
5. Use line below designated sample row to report cover for other species, surrounding habitat, and problems locating or accessing points.

Point #	Latitude	Longitude	A nodosum	F vesiculosus	Gracilaria	Ulva (foliose)	Ulva (tubular)	C sericea	P littoralis	C virgatum	C crispus	Vaucheria sp	S alterniflora	Z marina
BM141	-70.903489	43.058778												
Notes														
BM134	-70.905518	43.061147												
Notes														
BM029	-70.906949	43.059739												
Notes														
BM093	-70.906750	43.058499												
Notes														
BM159	-70.910402	43.054357												
Notes														
BM095	-70.911070	43.053698												
Notes														
BM047	-70.912988	43.050590												
Notes														
BM111	-70.916288	43.047630												
Notes														
BM031	-70.937127	43.033805												
Notes														

APPENDIX F. Results of stable isotope analysis performed by Boston University Stable Isotope Laboratory.

BOSTON UNIVERSITY STABLE ISOTOPE LABORATORY DATA SHEET

Date: 18 February 2014 — Samples Arrived: 11/12/13
Client: Elisabeth Cianciola — Job Number: 13CN63
Project: University of New Hampshire — File name: ci13CN63.xls
Data emailed: 2/18/14 — **Expected ^{13}C values:** — **Expected ^{15}N values:**
Data Location: Con 486 — **glycine -34.00** — **glycine 10.73**
peptone -14.73 — **peptone 7.40**

Tray Location	Sample	*CO_2 Ht. nA*	Wt (mg)	*%C*	δ^{13}C *(V-PDB)*	*N Ht. nA*	% N	δ^{15}N (air)
505 A-1	UNH 5	9.246	2.011	32.02	-13.68	4.580	2.88	7.73
505 A-2	UNH 6	10.159	1.976	36.05	-15.39	5.064	3.23	6.23
505 A-3	UNH 7	10.152	2.038	35.05	-16.45	5.267	3.24	5.94
505 A-4	UNH 7	9.867	1.981	35.10	-16.34	5.035	3.24	6.05
505 A-5	UNH 8	9.323	1.999	32.82	-15.24	4.053	2.58	6.70
505 A-6	UNH 9	10.333	1.965	37.00	-16.88	7.387	4.66	6.90
505 A-7	UNH 10	9.289	1.957	33.44	-16.08	6.665	4.29	8.39
505 A-8	UNH 10	9.485	1.982	33.72	-16.09	6.728	4.30	8.33
505 A-9	UNH 11	9.887	2.044	34.13	-16.39	6.878	4.22	8.41
505 A-10	UNH 12	9.640	2.005	33.82	-15.55	5.278	3.29	7.26
505 A-12	UNH 13	10.133	1.974	36.32	-9.68	5.379	3.43	8.74
505 B-1	UNH 13	10.363	1.995	36.73	-9.20	5.473	3.39	9.06
505 B-2	UNH 14	8.978	2.001	31.59	-11.11	4.106	2.56	8.92
505 B-3	UNH 15	8.704	1.988	30.89	-11.43	3.513	2.24	7.45
505 B-4	UNH 16	8.150	2.002	28.70	-10.84	4.004	2.55	7.33
505 B-5	UNH 16	8.349	2.016	29.21	-10.61	4.223	2.64	7.83
505 B-6	UNH 17	8.753	1.995	30.88	-10.78	4.046	2.55	8.31
505 B-7	UNH 18	9.716	1.995	34.42	-10.31	4.163	2.62	8.28
505 B-8	UNH 19	9.083	2.008	31.93	-11.50	2.459	1.59	7.04
505 B-9	UNH 19	9.051	2.001	31.87	-11.35	2.730	1.74	8.23
505 B-10	peptone	6.302	1.009	43.82	-14.87	11.745	14.92	7.15
505 B-11	UNH 20	9.352	1.977	33.39	-14.86	4.001	2.52	8.84
505 B-12	UNH 21	8.945	1.997	31.54	-15.60	5.526	3.47	6.55
505 C-1	UNH 22	8.911	1.995	31.47	-12.71	4.465	2.81	8.54
505 C-2	UNH 22	8.447	1.965	30.27	-12.41	3.802	2.47	8.02
505 C-3	UNH 23	8.892	1.968	31.87	-10.66	5.491	3.50	9.90
505 C-4	UNH 24	9.269	2.035	32.15	-10.81	5.203	3.23	8.43
	peptone	6.936	1.142	42.57	-14.71	13.248	14.74	7.48
	glycine	4.764	1.022	32.34	-33.84	14.901	18.62	10.61